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BIOLOGICAL BULLETIN

ON THE REVERSIBILITY OF THE HELIOTROPISM OF ARENICOLA LARVÆ BY CHEMICALS.[1]

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I. INTRODUCTORY.

Loeb discovered in 1893 that the normal heliotropism of *Polygordius* larvæ and marine copepods could be reversed by changes of the temperature and of the concentration of sea-water.[2] He also found in 1904 that fresh-water crustaceans which were naturally negative or indifferent to light could be made

[1] From the Marine Biological Laboratory, Woods Hole, Mass., and the Physiological Laboratory, University of Minnesota, Minneapolis.

[2] Loeb, Jacques, *Pflüger's Arch.*, Bd. 53, p. 81, 1893.

positively heliotropic by chemicals.[1] Since then, Holmes.[2] Minkiewicz,[3] Drzewina,[4] Jackson,[5] Moore,[6] Ewald,[7] and others have shown many examples of the reversibility of heliotropism in animals by chemicals, temperature, etc. R. S. Lillie,[8] Mast,[9] and the writer[10] have also observed the reversing effect of chemicals on the positive heliotropism of *Arenicola* larvæ. These observations, however, were rather incidental and not extensive,

In this paper, the writer shows quantitatively that the normal heliotropism of *Arenicola* larvæ can be reversed by various chemicals and by higher or lower temperatures than normal. And it will be seen that those chemicals which produce artificial parthenogenesis in sea-urchin and other eggs have also a reversing effect on the heliotropism of *Arenicola* larvæ. This parallelism between the reversal of the normal heliotropism of the larvæ and artificial parthenogenseis is striking as regards all chemicals used by the writer except inorganic acids.

The experimental work was done partly under the direction of Professor R. S. Lillie in the physiological department of the Marine Biological Laboratory at Woods Hole, Mass., U. S. A., during the summer of 1914. Publication has been delayed chiefly on account of the writer's return to Japan. The writer wishes here to acknowledge his indebtedness to Prof. Ralph S. Lillie and Prof. Elias P. Lyon for their valuable suggestions and criticism of the work and manuscript. His thanks are due also to Director Frank R. Lillie, who gave him the use of a research room in the laboratory.

II. Material and Methods.

The larvæ of the marine annelid, *Arenicola cristata*, were used for this work. The procedure of obtaining the larvæ was exactly

[1] Loeb, Jacques, Univ. of Calif. Pub., Physiol., Vol. 2, p. 1, 1904.
[2] Holmes, Samuel J., *Jour. Comp. Neur. and Psychol.*, Vol. 15, 305, 1905.
[3] Minkiewicz, R., *Arch. d. Zoöl. Exp. et Gén.*, t. 7, p. 37, 1907.
[4] Drzewina, Anna, *C. R. Soc. Biol.*, Vol. 71, 555.
[5] Jackson, H. H. T., *Jour. Comp. Neur. and Psychol.*, Vol. 20, p. 259, 1910.
[6] Moore, A. R., *Science*, N. S., Vol. 38, p. 131.
[7] Ewald, Wolfgang F., *Jour. Exp. Zoöl.*, Vol. 13, p. 597, 1912.
[8] Lillie, Ralph S., *Am. Jour. Physiol.*, Vol. 24, p. 14, 1909.
[9] Mast, Samuel O., "Light and the Behavior of Organisms, xi + 410 p., 1911.
[10] Kanda, Sakyo, *Am. Jour. Physiol.*, Vol. 35, p. 162, 1914.

the same as described in the writer's previous paper.[1] The free-swimming larvæ, just after they leave the egg-strings at the swarming stage, are strikingly positive to light. They are, therefore, very favorable for work of this kind.

The methods of experimentation were simple. At each trial, four 100 c.c. beakers, one for control with normal sea-water at room temperature, a second with a treated sea-water at room temperature, a third with the same (treated) sea-water at higher temperature, and a fourth with the same (treated) sea-water at lower temperature, were used. Fifty c.c. of liquid were used in each beaker. Each beaker was set in a larger vessel (finger bowl), containing sea-water of the same temperature as the beaker. When all the beakers were ready and fairly constant at the desired temperatures, the larvæ taken with a small pipette from a very dense aggregation were distributed as uniformly as possible in each beaker.

Diffuse light came horizontally from one south window only. Care was taken against reflected light by using folded black cloth under the beakers and on a long board that was placed at the north side of the dishes.

III. Experimental.

Recent discoveries in the reversing effect of temperature of normal sea-water on the normal heliotropism of various animals had made it desirable to examine this phenomenon in *Arenicola* larvæ and to see whether there is any temperature coefficient when chemicals were added to it. In some experiments this was fairly illustrated, as far as time-relation was concerned, but others were not so successful as expected, possibly on account of the entrance of other factors.

1. *Effects of Temperature.*

Mast states that he did not succeed in producing a reversal of the positive heliotropism of *Arenicola* larvæ by changing the temperature of sea-water.[2] This was not the case in the writer's experiments. Mast perhaps used old larvæ instead of very

[1] Kanda, *loc. cit.*

[2] Mast, *loc. cit.*

young ones. The sensitivity of the larvæ to changes of medium varied greatly according to their age. It was impossible to get larvæ uniformly young. This was the reason why one could not get a reversal of all larvæ treated even with the best agents. There was always one or two per cent. of exceptions. Table I. gives the summary of several series of the writer's experiments.

TABLE I.

EFFECTS OF TEMPERATURE.

50 C.c. Sea-water.	Immed. After the Treatment.	1 Min. After the Treatment.	5 Min. After the Treatment.	10 Min. After the Treatment.	25 Min. After the Treatment.
31° C.	Practically all positive	Some became negative.	About 50 per cent. became negative.	Same as before.	Same as before.
11° C.	Almost motionless.	Many became positive.	Very few became negative.	About 15 per cent. became negative.	About 20 per cent. became negative.
21° C. (Control)	Practically all positive.	Practically all on the window side	Same as before.	Same as before.	A few became negative.

It was peculiar that high and low temperatures had the same reversing effect on the larvæ, although cold was slower and less effective. Loeb[1] showed in *Polygordius* larvæ a phenomenon similar to this.

2. *Effects of Hypertonic and Hypotonic Sea-water.*

(*a*) *Effect of Hypertonic Sea-water.*—R. S. Lillie[2] and Mast[3] observed that hypertonic and hypotonic sea-water produced the reversal of the normal heliotropism in *Arenicola* larvæ. The writer tested the effect of hypertonic sea-water by mixing 15 c.c of one mol NaCl solution and 35 c.c. of natural sea-water at room temperature, 22° C. He found that about 20 per cent. of larvæ became negative. He did not, however, succeed in producing a reversal in this medium by raising or lowering temperature, *i. e.*, 32° C. or 12° C. The larvæ in this mixture were very sluggish at these temperatures.

In the mixture of 0.85 c.c. of m-KCl solution and 50 c.c. of

[1] Loeb, J., *loc. cit.*

[2] Lillie, R. S., *loc. cit.*

[3] Mast, S. O., *loc. cit.*

natural sea-water at room temperature, 22° C., it was found that 30 minutes after the treatment about 35 per cent. of the larvæ became negative to light. Besides sodium and potassium chlorides, m-$CaCl_2$, m-$MgCl_2$, and 2m-$MgSO_4$ were tested without success. Temperature experiments also failed in all these mixtures of hypertonic sea-water.

(*b*) *Effect of Hypotonic Sea-water.*—The mixture of 30 c.c. of natural sea-water and 20 c.c. of distilled water (which was the optimum dilution) was very remarkable in producing a reversal of heliotropism. The effect was temporary only. The results of several series are summarized in Table II. It is also interesting to note the difference in the time required to produce the maximum results at the three temperatures 12°, 22°, 32°. A Q_{10} value of about 2 is indicated for the reversive process.

TABLE II.

EFFECT OF HYPOTONIC SEA-WATER.

Solution.	Temperature.	Best Result Obtained.	Result.
30 c.c. sea-water + 20 c.c. dist. water.	32° C.	10 min. after the treatment.	About 90 per cent. became negative.
30 c.c. sea-water + 20 c.c. dist. water.	22° C. (room temp.)	20 min. after the treatment.	About 90 per cent. became negative.
30 c.c. sea-water + 20 c.c. dist. water.	12° C.	40 min. after the treatment.	About 90 per cent. became negative.
50 c.c. nat. sea-water (control).	22° C. (room temp.)	1/2 min. after the treatment.	Practically all positive.

The larvæ lived over 15 days and grew well in this hypotonic sea-water.

3. *Effects of Isotonic Salt Solutions.*

A question arose whether these reversing effects of hypertonic and hypotonic sea-water were simply osmotic or not. This idea was tested by using isotonic salt solutions. In all of these series the problem of temperature was omitted from consideration. The results are given in Table III.

Since the sodium, lithium, potassium or ammonium chloride and sulphate solutions that were used were all isotonic with sea-water, it is evident that the reversing effect of these salts is not simply an osmotic one. And since the action of these eight salts was different from that of calcium or magnesium chloride

TABLE III.

EFFECT OF ISOTONIC SALT SOLUTIONS.

Temp. of Mixt.	Mixture of Isot. Sol. and Sea-water.	Time After the Treatment for Maximum Effect.	Per Cent. of Negative Heliotropism Produced.
20° C.	1.5 c.c. 0.52 m. NH_4Cl + 50 c.c. sea-water.	About 1 hr.	About 90 per cent.
20° C.	2 c.c. 0.52 m. KCl + 50 c.c. sea-water.	About 20 min.	About 70 per cent.
21° C.	3 c.c. 0.54 m. LiCl + 50 c.c. sea-water.	About 25 min.	About 50 per cent.
21° C.	30 c.c. 0.52 m. NaCl + 20 c.c. sea-water.	About 1 hr.	About 30 per cent.
21° C.	20 c.c. 0.35 m. $MgCl_2$ + 30 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
21° C.	20 c.c. 0.35 m. $CaCl_2$ + 30 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
20° C.	1 c.c. 0.46 m. $(NH_4)_2SO_4$ + 50 c.c. sea-water.	About 2 hrs.	About 90 per cent.
20° C.	1 c.c. 0.46 m. K_2SO_4 + 50 c.c. sea-water.	About 40 min.	About 75 per cent.
21° C.	1.5 c.c. 0.47 m. Li_2SO_4 + 50 c.c. sea-water.	About 1 hr.	About 70 per cent.
21° C.	20 c.c. 0.46 m. Na_2SO_4 + 50 c.c. sea-water.	About 2 hrs.	About 60 per cent.
21° C.	12 c.c. 0.9 m. $MgSO_4$ + 50 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
21° C.	50 c.c. nat. sea-water.	About 1/2 m.–5 hrs.	Pract. all positive.

and sulphate solutions, the reversing effect of the former eight salts would seem to be specific. The chlorine and sulphate ions in these twelve salts being the same, the specific action of isotonic sodium, lithium, potassium or ammonium chloride and sulphate solutions seems to be due essentially to cations instead of anions. The effective order of these cations may be expressed as follows:

$$Na^+ < Li^+ < K^+ < NH_4^+$$

That a yellow pigment contained in the cells of the larvæ diffused out into the medium, when treated with the mixture of isotonic Na_2SO_4 or NH_4Cl solution and sea-water, was striking. This took place between eight and ten minutes after the treatment A similar exit of pigment was first observed by Lillie in isotonic NaCl, KCl and NH_4Cl solutions, and has been generally regarded as indicating an increased permeability of the cell membrane.

A question arises whether the reversal of positive heliotropism of the larvæ was due directly to the diffusion of cations into the cells as indicated above or not. In the cases of Na_2SO_4 and

NH_4Cl solutions, the most notable exit of pigment from the larvæ—the sign of increased permeability according to Lillie—took place at about ten or twenty minutes after the treatment. In the case of Na_2SO_4 solution about 5 per cent. of the larvæ became negative at about ten minutes, while in NH_4Cl solution the larvæ were evenly distributed at about 20 minutes after the treatment. The maximum reversal in the former was produced at about two hours and in the latter at about one hour after the treatment. The reversing effect of these two salts, therefore, seems to be too slow to be regarded as the direct action of ions which have diffused into the larvæ. At the same time, the effect is not on the boundary membrane alone, because these salts must have modified and penetrated the membrane long before the change of reaction in the larvæ occurred. The larvæ treated with Na_2SO_4 and NH_4Cl stick tightly to the window side of a beaker 7 or 8 minutes after the treatment. This fact may explain the difficulty. That is to say, if they had been free in swimming, they might have reversed their heliotropic reaction more promptly than they actually did.

As to the way in which the behavior of organisms is modified by electrolytes such as salts, we are as yet in the dark. At any rate, the reversal of positive heliotropism of the larvæ by these electrolytes is an insufficient index by which to gain an understanding of the mechanism of their physical or chemical influence on the behavior of organisms.

4. *Effects of Artificial Sea-water.*

The artificial sea-water that the writer used was a van't Hoff mixture of the following composition:

0.52 m. Nacl	100 c.c.
0.52 m. KCl	2.2 c.c.
0.35 m. $CaCl_2$	1.5 c.c.
0.35 m. $MgCl_2$	7.8 c.c.
0.90 m. $MgSO_4$	3.8 c.c.

To this a trace of $NaHCO_3$ was added as Loeb advised. This artificial sea-water with or without the alkali affected little the positive heliotropism of the larvæ. About five per cent. of them showed negative heliotropism. Besides these, a few larvæ in

this solution swam about at the bottom of the beaker. About 90 per cent. or more of the larvæ were always positive to light.

5. *Effects of Hypertonic Sodium and Potassium Chloride Solutions Added to the Artificial Sea-water.*

These effects were remarkable. In the mixture of 15 c.c. m-NaCl and 35 c.c. of the artificial sea-water, about 90 per cent. of the larvæ became negative to light at about 20 minutes after the treatment. Most of the negative animals stayed at the bottom of the negative side of the beaker. In the mixture of 0.8 c.c. of m-KCl and 50 c.c. of the artificial sea-water, which was the optimum proportion, about 75 per cent. of the larvæ became negative to light at about 30 minutes after the treatment.

The mixture of 15 c.c. of isotonic NaCl and 35 c.c. of the artificial sea-water was also tested. It changed the reaction of about 85 per cent. of the larvæ. In comparing these results with those shown in Table III., it may be concluded that the effective order of ions is variable as other environmental conditions are varied. It is little wonder therefore that the order of ionic actions obtained by different investigators is not always uniform.

6. *Effects of Elimination of Certain Salts from the Artificial Sea-water.*

By eliminating each separate component from the artificial sea-water made up of six components, the specific rôle which such component played on either positive or negative heliotropism of the larvæ might be, the writer thought, in part understood.

First of all, NaCl solution was left out of the artificial sea-water. Larvæ transferred into the mixture without NaCl at 22° C., were practically all motionless immediately after the treatment. Sodium chloride being the essential component of the artificial sea-water as well as of the natural one, such result should be expected, since the osmotic as well as the chemical conditions are profoundly changed.

According to Ewald, however, "leaving NaCl out of artificial sea-water" "increases the negative and diminishes the positive" heliotropism of the nauplii of *Balanus perforatus* which are

naturally positive to light. Such was not the case in *Arenicola* larvæ, as just stated.

Elimination of KCl had a different effect. After transferring the larvæ into the artificial KCl-free sea-water at 23° C., practically all showed positive reaction immediately after the treatment, though a few soon became negative. About 20 minutes after the treatment, about 20 per cent. of all larvæ were at the top of the positive side of the beaker, about 30 per cent. at the bottom of the same side, and about 50 per cent. at the bottom of the negative side. If the larvæ collected at the bottom of the beaker farthest from the source of light could be called negative, one sees a puzzle hardly capable of solution. For, as already seen, addition of 2 c.c. of isotonic KCl solution to 50 c.c. of the of the natural sea-water also reversed about 70 per cent. of the larvæ. In other words, either addition or elimination of potassium salt produced a negativating effect on the larvæ. Besides potassium, however, there was another negativating component in the artificial sea-water, namely sodium. In the unbalanced artificial sea-water, sodium ions might exert a negativating effect on the larvæ.

In the calcium-free artificial sea-water at 23° C. all larvæ swam about at the bottom of the beaker. Their activity was much diminished; and about 15 minutes after the treatment a majority of them became almost motionless. $CaCl_2$ in the artificial sea-water as well as in the natural one is therefore essential to the life and activity of the larvæ, although of the five salts it is present in the smallest proportion. It is well known that calcium and magnesium antagonize the toxic action of sodium and potassium. Magnesium alone seemed, however, not to be sufficient in this case.

In the magnesium chloride-free or sulphate-free artificial sea-water at 23° C., immediately after the treatment practically all the larvæ were positive. About 10 minutes after the treatment about 60 per cent. or more of them were positive, about 20 per cent. negative, mostly at the bottom of the negative side of the beaker, and 20 per cent. or less were scattered at the bottom. During 1 to 5 hours after the treatment about 70 per cent. or less were positive, and the rest were at the bottom of the negative

side. When both $MgCl_2$ and $MgSO_4$ were left out at 23° C., those larvæ which were at the bottom of the negative side constituted about 40 per cent. or more, whereas they were about 30 per cent. or more with elimination of either $MgCl_2$ or $MgSO_4$ alone. As has already been seen, the addition of magnesium chloride or sulphate to the natural sea-water had no negativating effect on the heliotropism of the larvæ. On the other hand, the addition of sodium or potassium chloride had such effect. The fact that magnesium and calcium chlorides seem to act as a positivating component in the artificial sea-water as well as in the natural sea-water is therefore clear, whereas potassium and sodium chlorides act as negativating ones. In other words, magnesium and calcium chlorides act in antagonizing the negativating effect of sodium or potassium chloride, or of both, although magnesium cannot antagonize the toxic action of the latter salts as calcium chloride can do, since the larvæ could not remain active and live long in calcium-free artificial sea-water.

7. *Effects of Alcohols.*

In narcotising *Arenicola* larvæ, Lillie used many narcotics. Of alcohols, he found that ethyl alcohol had a negativating effect on the heliotropism of the larvæ. It will be seen in Table IV. that all of the alcohols tested by the writer had more or less effect in making the larvæ negative to light. Difficulty was experienced, however, in getting uniform results in different series of experiments when different stocks of alcohols were used; probably this variability was largely due to impurity of reagents.

Just as Loeb has shown with copepods and *Daphnia*, it is obvious from Table IV. that the higher the alcohol in the series the greater negativating effect it had. This point will be considered a little later. With the exception of two cases, the higher the temperature the quicker and larger was the negativating effect produced. That the larvæ in the mixture of methyl or ethyl alcohol and natural sea-water at room temperature (22°) were less negative than at a temperature five degrees lower (17°) is hard to understand.

Acetone and two esters were also tested. The general features of the results obtained with these narcotics were quite similar to

TABLE IV.

EFFECTS OF ALCOHOLS.

Concentration of Alcohol Solution (made with Sea-water).	Temperature.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.		
		10–15 Min.	20–25 Min.	30 Min.
0.03 m. methyl alcohol.	32° C. 22° C.(R.T.) 17° C.	65 per cent.	15 per cent.	30 per cent.
0.016 m. ethyl alcohol.	32° C. 22° C.(R.T.) 17° C.	65 per cent.	25 per cent.	30 per cent.
0.002 m. n-propyl alcohol.	32° C. 22° C.(R.T.) 17° C.	70 per cent.	45 per cent.	35 per cent.
0.0006 m. n-butyl alcohol.	31° C. 21° C.(R.T.) 16° C.	70 per cent.	45 per cent.	35 per cent.

those obtained with alcohols. In no case, however, were the larvæ normal in behavior when the temperature was lowered either 5° C. or 10° C. below room temperature. On the other hand, production of negative heliotropism at the higher temperature (32°) was much more striking with the esters than with any of the alcohols. Table V. gives a summary of these series of experiments.

TABLE V.

EFFECTS OF ACETONE AND ESTERS.

Concentration of Narcotic Made with Sea-water.	Temperature.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.	
		10 Min.	20 Min.
0.01 m. acetone.	32° C. 22° C. (R.T.)	40 per cent.	25 per cent.
0.0045 m. ethyl acetate.	32° C. 22° C. (R.T.)	90 per cent.	30 per cent.
0.0025 m. ethyl acetate.	32° C. 22° C. (R.T.)	100 per cent.	45 per cent.

From Tables IV. and V. nothing certain can be concluded as to the temperature coefficient in the production of negative heliotropism, although the time needed to reach the maximum point is about halved by a rise of 10° C.

8. *Effect of Saponin.*

A solution of saponin made by adding 0.8 c.c. of 0.1 per cent. saponin to 50 c.c. of natural sea-water was also tried. The influence of temperature was well marked. At room temperature about 10 per cent. of the larvæ became negative after 30–40 minutes in this solution: at 10° above room temperature about 90 per cent. became negative within 20 minutes.

9. *Effects of Fatty Acids.*

"Loeb hat . . . quantitative Versuche über die zur Erzeugung von positivem Heliotropismus bei Daphnien und Copepoden nötige Konzentration verschiedener Säuren und Alkohole gemacht. Die folgende Tabelle gibt die minimale Konzentration für die Erregung von positivem Heliotropismus bei Copepoden für verschiedene Säuren und Alkohole:

Ameisensäure	0.006	N.
Essigsäure	0.006	"
Propionsäure	0.005	"
Buttersäure	0.004	"
Valeriansäure	0.004	"
Capronsäure	0.0019	"
Aethylalkohol	0.19	"
Propylalkohol	0.054	"
Normaler Butylalkohol	0.019	"
Amylalkohol	0.011	"

Man sieht, dass die Wirksamkeit der Alkohole sehr rasch mit steigender Zahl der Kohlenstoffatome zunimmt, während bei den Säuren der Zuwachs nur eben merklich ist. Die Folge ist, dass, während die niedrigen Alkohole viel weniger wirksam sind als die korrespondierenden Fettsäuren, bei den höheren Gliedern dieser Unterschied verschwindet." [1]

In comparing the results obtained by Loeb with those which are given in Tables IV. and VI. a general agreement will be found. It is also worth mentioning that the reversal of the larvæ in acidified sea-water took place immediately after the treatment at a temperature 10° C. higher than room temperature, whereas it took place about two minutes after the treatment at room temperature. In this experiment and others shown in

[1] Loeb, Jacques, "Handbuch d. Vergl. Physiol.," Bd. IV., Erste Hälfte, S. 470.

Table VI., the temperature coefficient (Q_{10}) of the maximum reversal of the normal heliotropism appeared to be between 2 and 3. The reversal may, therefore, be interpreted as the result of a chemical reaction. The marked physiological activity of the fatty acids seems to depend largely on their lipoid-solubility. At lowered temperatures their effectiveness in producing negative heliotropism is somewhat lessened, as Table VI. shows.

TABLE VI.

EFFECTS OF FATTY ACIDS.

Concentration of Fatty Acid Solution (Made with Sea-water).	Temperature, Degrees C.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.		
		5 Min.	10–15 Min.	20–30 Min.
0.0025 m. formic acid.	32	90 per cent.		
	22 (R.T.)		85 per cent.	
	12			20 per cent.
0.0010 m. acetic acid.	32	95 per cent.		
	22 (R.T.)		90 per cent.	
	12			30 per cent.
0.0015 m. propionic acid.	32	95 per cent.		
	22 (R.T.)		90 per cent.	
	12			60 per cent.
0.0011 m. butyric acid.	32	95 per cent.		
	22 (R.T.)		95 per cent.	
	12			90 per cent.
0.00075 m. valeric acid.	31½	95 per cent.		
	21½ (R.T.)		95 per cent.	
	11½			20 per cent.
0.00006 m. caproic acid.	31½	95 per cent.		
	21½ (R.T.)		95 per cent.	
	11½			

Of all chemicals tested by the writer, fatty acids were the best for reversing normal heliotropism. If one reversed the heliotropism of a mass of larvæ by fatty acid, a large percentage gradually accumulated at the side of the dish away from the light. If now one turned the dish through 180°, it was most striking to see the hosts of larvæ quickly assume the new orientation and swim to the negative side. Their general behavior and activity appeared normal. Many, however, swam at the bottom of the negative side of the dish, especially in solutions of the higher fatty acids. Curiously enough, this efficiency of fatty acids as a reverser of heliotropism runs closely parallel to their physiological efficiency in producing artificial parthenogenesis, as discovered by Loeb.

10. *"Antagonism" Between Acid and Narcotics.*

It is known through the work of R. Lillie that an antagonism between salts and narcotics is readily demonstrated in *Arenicola* larvæ. In the present investigation it was found that chloroform, ethyl alcohol and ether had little or no negativating effect on the larvæ at room temperature; on the other hand, butyric acid proved to be the most effective of all the negativating agents employed. The writer thought therefore that antagonism might be demonstrated in the mixture of any of those narcotics and butyric acid. It was found that the negativating effect of butyric acid was much retarded in the presence of a narcotic. In sea-water containing .0022 m. butyric acid 95 per cent. of the larvæ became negative after 15 minutes exposure. In the same solution to which chloroform, ether, or formaldehyde were added in appropriate concentrations no reversal was evident after 15 minutes and only 50 per cent. were negative after one hour's exposure, as compared with 90–100 per cent. in the control. Apparently the process of reversal is retarded in the presence of the anæsthetic.

11. *Effect of Solutions of Butyric Acid made with Artificial Sea-water.*

The writer tested the effect of butyric acid in artificial sea-water. It was found that a very weak concentration of butyric acid, *i. e.*, 0.00006 mol., was efficient in negativating about 80 per cent. of the larvæ in about 10 minutes after the treatment.

12. *Effects of Inorganic Acids.*

Loeb[1] found that the monobasic fatty acids were very effective, whereas strong acids, such as HCl, HNO_3 and H_2SO_4 "had so little effect as to be practically useless" for experiments on the membrane formation of sea-urchin eggs in artificial parthenogenesis. He concludes that this proves a diffusion of undissociated molecules into cells and denies an increased permeability of the cells for hydrogen ions.[2] The writer tested this idea in producing the negative heliotropism of larvæ, but the results

[1] Loeb, Jacques, *Biochem. Zeits.*, Bd. 15, S. 254, 1909; "Artificial Parthenogenesis and Fertilization," p. 133, 1913.

[2] Loeb, J., *loc. cit.*, pp. 142–143.

obtained by the writer did not agree well with those of Loeb, as in the case of fatty acids. For instance, in 0.0036 m. HCl solution made with sea-water, about 80 per cent. of the larvæ became negative and in similar solutions of H_2SO_4 about 95 per cent. became negative 15 minutes after the treatment at 21° C. The negative larvæ, however, showed marked abnormality. This was the only case of failure, so far as the writer found, in the parallelism between the effects of chemical substances in producing artificial parthenogenesis in sea-urchin eggs and in reversing the positive heliotropism of *Arenicola* larvæ.

The writer also found at the Marine Biological Laboratory of the Kyushu Imperial University, Fukuoka, Japan, in the summer of 1916, that a marine crustacean, *Astracoda Cypridina (hilgendorfi)* which was strongly negative to light, could be made positive by HCl or H_2SO_4 solution. Quantitative data were not obtained owing to illness.

That acids, organic and inorganic, are able to penetrate the living cell, is proved by Harvey.[3] It seems possible therefore that both HCl and H_2SO_4 penetrated into *Arenicola* larvæ.

13. *Effects of Strong and Weak Bases.*

Loeb discovered that "the weak base NH_4OH is much more efficient for the causation of artificial parthenogenesis in *Arbacia* eggs than the strong bases, NaOH, KOH and tetraethylammonium hydroxide,"[2] $N(C_2H_5)_4OH$. The writer found that this was also the case in the production of negative heliotropism in *Arenicola* larvæ. The essential results of his experiments may be summarized thus: in the appropriate concentration of NH_4OH, about 70 per cent. of the larvæ became negative after about 20 minutes exposure to the solution at 21° C., whereas in equivalent solutions of NaOH, KOH, or $N(C_2H_5)_4OH$, only about 10 or 15 per cent. of the larvæ became negative after an exposure of about 60 minutes at this temperature. This difference may be explained by the fact that the living cell is permeable to ammonium hydroxide but impermeable to sodium hydroxide and other strong bases, as experiments of Bethe[3] and Warburg[4] have

[1] Harvey, E. Newton, *Science*, N. S., Vol. 39, p. 947, 1914.

[2] Loeb, Jacques, *Jour. Ex. Zoöl.*, Vol. 13, p. 577, 1912.

[3] Bethe, Albrecht, *Pflüger's Arch.*, Bd. 127, S. 219, 1909.

[4] Warburg, Otto, *Zeits. physiol. Chem.*, Bd. 66, S. 305, 1910.

clearly shown. Possibly the weak base NH_4OH is more effective than the stronger bases because of greater solubility in the lipoids of the plasma membranes.

14. *Effects of Chemicals after Returning into Normal Sea-water.*

Lillie observed one curious effect in larvæ treated with one fourth and one fifth saturated solutions of chloroform in sea water; when the larvæ, after about two hours in corked flasks containing these solutions, were poured out into watch glasses the great majority were found, after three or four hours (when the chloroform had evaporated), to exhibit a pronounced negative phototaxis in place of the usual positive. . . .[1] As was pointed out in his previous paper, the writer found a similar phenomenon in the larvæ treated with sodium, potassium, calcium and magnesium chloride solutions when they were returned into normal sea-water.[2] This was particularly remarkable in the larvæ treated with the last two solutions during the actual immersion in which there was never any reversal of positive heliotropism.

In the present experiments, the same phenomenon was also found in larvæ treated with salts, narcotics—chloroform, chloral and formalin in particular—strong alkalies, potassium cyanide, etc. As already shown, most of these chemicals were not favorable agents for the reversal of the positive heliotropism of the larvæ. Moreover, in some cases, KCN, for instance, the larvæ remained positive or indifferent to light while in presence of the chemical substance (dissolved in normal sea-water). Nevertheless the "after-effect" was pronounced. That is to say, almost all larvæ became negative when returned to normal sea-water.

Such facts are consistent with the hypothesis that these chemicals act by changing in some way the normal properties of the plasma membranes of the irritable elements concerned, so that a certain time is required to recover normal conditions after return to normal sea-water.

[1] Lillie, R. S., *loc. cit.*, p. 34.
[2] Kanda, S., *loc. cit.*
[3] Lillie, R. S., *loc. cit.*, p. 34.

IV. Summary.

1. At room temperature nearly all *Arenicola* larvæ in the early swarming stage in normal sea-water are positively heliotropic. At a temperature 10° C. higher (30°–34°) about half of the larvæ become negative to light after about 5 minutes' exposure. At 10° C. lower than room temperature (ca. 12°), about 20 per cent. of the larvæ become negative after about 25 minutes exposure.

2. In sea-water made hypertonic by addition of NaCl or KCl some larvæ (20–35 per cent.) become negative.

3. In sea-water made hypertonic with $CaCl_2$, $MgCl_2$ or $MgSO_4$, no reversal was observed.

4. In hypotonic sea-water, about 90 per cent. of the larvæ reversed their heliotropism; reversal takes place more rapidly the higher the temperature (between 12° and 32°).

5. Isotonic solutions of ammonium, potassium, lithium, sodium chlorides and sulphates made in sea-water reverse the positive heliotropism of the larvæ. But isotonic solutions of magnesium and calcium chlorides and sulphates in sea-water do not. The reversing effect of these salts seems, therefore, due to the cations. This suggests an action on the plasma membranes (*cf.* the membrane theory of Ostwald-Bernstein).[1] The effective order of these cations is

$$Na^+ < Li^+ < K^+ < NH_4^+$$

6. In artificial sea-water, the positive heliotropism of the larvæ is not noticeably different from that in natural sea-water. A trace of alkali makes apparently no difference.

7. Hypertonic sodium and potassium chloride solutions added to the artificial sea-water reversed the positive heliotropism of the majority of the larvæ.

8. In sodium-free artificial sea-water, the larvæ became motionless immediately after the treatment (effect of hypotony).

9. In potassium-free artificial sea-water, many became negative to light, and remained at the bottom of the negative side of the beaker.

10. In calcium-free artificial sea-water, the larvæ became motionless about 15 minutes after the exposure. Magnesium

[1] Bernstein, J., *Pflüger's Arch.*, Bd. 122, S. 129.

alone, therefore, cannot antagonize the toxic action of sodium and potassium salts.

11. In the magnesium-free artificial sea-water, many remain positive to light. This fact may be explained as an antagonistic action of calcium toward sodium and potassium salts which acting by themselves are negativating agents.

12. Alcohols (methyl, ethyl, n-propyl, n-butyl) as used by the writer are not favorable as negativating agents at room temperature, though they are good at higher temperature. The higher the alcohol in the series the greater negativating effect it has.

13. Most of the larvæ treated with esters, methyl and ethyl acetate, became negative at higher than room temperature.

14. Other compounds, chloroform, formalin, ether and saponin, produced little or no reversal while the larvæ were in the solutions, except saponin at higher temperatures.

15. Monobasic fatty acids are the best of all chemicals used for this work. The higher the acid in the series the greater negativating effect it has.

16. Some narcotics "antagonize" or retard the reversing action of butyric acid.

17. The reversing effect of a very weak concentration of butyric acid (.00006 m.) in artificial sea-water is great.

18. Hydrochloric and sulphuric acids produce negative heliotropism, almost as well as fatty acids do.

19. In producing negative heliotropism, NH_4OH is much more efficient than strong bases.

20. *Arenicola* larvæ treated with narcotics become negative after returning into normal sea-water.

THE RELATION OF PLUMAGE TO OVARIAN CONDITION IN A BARRED PLYMOUTH ROCK PULLET.[1]

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The assumption by female birds of plumage and other external characters which ordinarily distinguish the male is not an uncommon phenomenon and has long been noted. While it is known to occur among wild birds (Hunter, 1780, p. 532), it has been much more commonly observed in domesticated varieties or others retained in captivity, where the opportunity for close observation is obviously much greater. An excellent general summary of the literature on the subject, including a most useful bibliography, has recently been published by Larcher (1916).

Cases have most frequently been reported in fowls, pheasants and ducks, and it is probable that most poultrymen of wide experience have known of their occurrence. A careful search of the poultry journals would doubtless reveal mention of many cases, though the proportion which gets into print must be relatively small, and the number that receive scientific attention even smaller. One such has been reported recently (Dent, 1917) of a mongrel Barred Plymouth Rock which was claimed not only to have laid eggs but also to have treaded hens. It was stated, furthermore, that the eggs from these hens with which the hermaphrodite mated were successfully hatched. The bird in question was also said to crow at times and to sing like a hen every day. A photograph accompanying the letter shows a bird with a head like a cock, but body more resembling a hen. If confirmed, this would be an extreme case of hermaphroditism.

[1] Contribution from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 14, and from the Kansas Agricultural Experiment Station. Published with the approval of the Directors.

An extensive study of a number of similar, though less extreme cases, has just appeared (Boring and Pearl, 1918), and these will be mentioned later in connection with our own case.

In the foregoing cases the non-functioning of the ovary and the consequent deficiency of ovarian secretion was due to natural causes, probably in most instances to a diseased condition of the ovary. The deficiency of ovarian secretion can also be brought about by artificial removal of the ovary. This is a delicate operation but has been successfully accomplished and the effects on ovariotomized birds have been reported by Guthrie (1910) on a hen, Fitzsimons (1912) on ostriches, and Goodale (1913, 1916*a*, 1916*b*) on chickens and ducks. In all cases more or less complete assumption of characteristic male plumage, and in varying degrees of other masculine characters, appeared after the ovaries were removed, some individuals, as Goodale says, becoming "nearly complete replicas of the male."

The subject of the present note was a purebred Barred Plymouth Rock pullet. She was hatched on the poultry farm of the Kansas State Agricultural College in 1915 and received the number 1050, as a consequence of which she has since been designated in our records as "Kansas 1050." In the early months of her life she was not noticeably different from her sisters and flock mates, and it therefore happens that no photograph was taken of her during that time. In February, 1916, she was put into a breeding pen as a normal pullet, along with others, but some time later it was noticed that she was taking on marked male characteristics, particularly with respect to plumage. Fig. 1 shows her appearance in April, 1916, while Fig. 2 is one of her normal flock sisters at about the same time. For purposes of comparison a photograph (Fig. 3) is also introduced of a normal cockerel, a full brother of the pullet shown in Fig. 2. A comparison of these photographs shows even better than words can describe the character relations of the three individuals. It will be seen that "Kansas 1050" has almost perfect male plumage, except that the sickle feathers of the tail are not so long and the breast and under parts are slightly darker, due to the fact that the white bars on the feathers are somewhat narrower in proportion to the black. The characteristic difference

in barring in males and females of the same strain is illustrated in Figs. 2 and 3. This will be discussed later.

The change in plumage between February and April denotes of course, that a molt occurred during this period. As the normal season for molting is in the fall, this is in itself a matter of interest and implies some underlying physiological process. In view of the close relation well known to exist between molting and ovarian activities, it seems reasonable to suppose that the bringing on of the molt at an unusual season, as well as the modification of the character of the new plumage, was due to changes in the ovary induced by the development of the pathological condition to be described later. While the effect on the character of the plumage is very likely the direct result of the action of changed internal secretions from the sex gland, it seems more probable that the molt is a secondary effect due to the change in metabolism, since in general molting seems to be most closely linked to metabolic conditions of the individual. This is indicated by the fact that molting may at times be induced by other conditions than changed sex-gland activity, such as change of feed, injury, and other environmental influences.

In other respects "Kansas 1050" was at that time rather intermediate between the normal pullet and cockerel. The head and comb were more like that of the pullet; shape and carriage of the body were intermediate. Spurs were not developed. In general she may be said to have looked much like a capon, a resemblance which was noted by a number of observers.

"Kansas 1050" was brought to Madison in the fall of 1916 and was merely kept under observation all of that winter. Her behavior during that time showed nothing of special interest, and in so far as indications of sexuality were concerned was practically indifferent. She seemed in excellent health and "sang" a great deal, somewhat resembling a hen in this respect, except that the voice had a peculiar more or less masculine quality. She was never heard to crow. She showed no indications of laying, and it was suspected that she probably had an ovarian tumor, which would account also for the plumage change. Thinking that possibly the accessory sexual organs might be

normal even though the ovary was affected, we decided to try the experiment of removing the tumor and of ingrafting an ovary from another individual, in order to see what effect it would have on the secondary sexual characters, and also in the hope that eggs might be produced normally from the engrafted ovary.

The operation was undertaken on April 8, 1917, an incision being made in the usual place for caponizing. This disclosed an enormous tumor, estimated to be some 10 cm. in diameter, and highly vascular on the surface. It seemed probable that the tumor was of ovarian origin, but on account of its size its point of attachment could not be definitely determined. Its removal was, moreover, deemed impracticable because of the highly developed and ramifying vascularization. Consequently several pieces of ovary from a freshly killed "white" Andalusian pullet were inserted loosely into the body cavity, and the wound closed. This pullet was nearly ready to lay, an ovum having already entered the oviduct; but only pieces of the ovary with very small ova were used for transplantation.

The patient made an uneventful recovery, except that the ether used as the anæsthetic apparently caused some irritation of the air passages, resulting in a slight "rattling" in the breathing, which persisted for some time. General health, however, appeared excellent, and the bird was bright and active.

On May 3 a white Wyandotte cock was put into the pen with "Kansas 1050" to test their behavior. He showed no antagonism to "Kansas 1050" but treated her as if she were a female, in spite of her male appearance. She on her part behaved more like a female, showing no fear of the cock and coming at his food call. She did not, however, display any signs of sexual activity.

On this date new feathers were beginning to come in on the area which had been plucked for the operation, and as they seemed to differ somewhat in character from the old feathers, other areas were denuded in order to make a further test of the matter. For this purpose the hackle, saddle and sickle feathers were plucked from the left side of the body, leaving those on the right side in place. By July 28, when the photographs reproduced in Figs. 4 and 5 were taken, the feathers on the neck and

lower back had been entirely replaced, but new tail feathers had not yet grown out. Fig. 4 shows distinctly the difference in the old feathers on the right side of the neck and the new feathers on the left side. The difference is even more marked on the lower back, as shown in Fig. 5. In both locations the new feathers on the left appear darker and are distinctly hen-feathers in shape while the older feathers of the right side are lighter and elongated like those of a cock. A careful comparison of the two sets of feathers was made to ascertain how closely they resembled normal feathers of the two sexes respectively.

The American Standard of Perfection[1] stipulates that in the ideal of the Barred Plymouth Rock breed, "the light and dark bars [are] to be of equal width, in number proportionate to length of feathers, and to extend throughout the length of feathers in all sections of the fowl." Every breeder who has had any experience with this breed of fowls knows that this standard is a purely artificial one, for in any particular strain the males always run lighter in color than the females (compare Figs. 2 and 3) there being, as Pearl and Surface (1910, p. 47) remark, "actually a sexual dimorphism in respect to plumage color in Barred Plymouth Rocks as they are ordinarily bred." This has driven the fancier to a system of breeding commonly known as "double mating," but which really means the carrying along side-by-side of what are virtually two sub-varieties of the breed, one in which the pullets are very dark and the cockerels approach the Standard requirements, and the other in which the pullets are about medium and the cockerels much lighter. For showing in the Standard classes males are chosen from the former line and females to match them from the latter. The dark "cockerel bred" pullets and the light "pullet bred" cockerels are sometimes shown in entirely distinct classes as breeding stock. The males and females of Standard color are not bred together, but each is mated to selected birds of its own breeding.

This fact was commented on a number of years ago by Spillman (1909), who suggested it would be wiser for the fancier to endeavor to learn just the shade of difference which "nature tries to produce between the sexes and then change the Standard

[1] Amer. Poult. Assn., 1915, p. 50.

accordingly." His explanation of the natural difference in shade in the two sexes is that the males are homozygous whereas the females are heterozygous with respect to barring. "The males, therefore, have twice as much tendency to barring as do the females. The males are therefore more barred than the females and hence are lighter in color." By "more barred" Spillman means that the white bars are wider in proportion to the black. This difference in the relative widths of the white and black bands on the feathers may be noted in the normal cockerel and pullet, Figs. 2 and 3, but is more evident in the individual feathers shown in Figs. 7–10. These feathers, while not from the same individuals shown in Figs. 2 and 3, are from birds of the same breeding as these and "Kansas 1050." Fig. 7 is a neck feather from hen 804, which was a flock sister of "Kansas 1050," while Fig. 8 is a feather from the cushion, or lower back, of the same bird. Fig. 9 represents a hackle feather, as it is called in the male, from the neck of a cock bird, 104E, which is the son of a flock sister of "Kansas 1050." Fig. 10 is a saddle feather from the same male, being from the position corresponding to the cushion in the hen. It will be noticed that in addition to the differences in shape in the two sexes, the white and black bars on the hen's feathers are of practically the same width, while on those of the cock the white bars average considerably wider.

From the photograph of the neck feathers of "Kansas 1050" in Fig. 4 the impression is gained that the masculine feathers of the right side have broader white bars; but that such is not the case on the lower back is evident from a careful inspection of Fig. 5. Furthermore, the fact that the white bars are of the same width in the old and new feathers of both these regions is convincingly shown in Figs. 11–14. Figs. 11 and 12 are neck and lower back feathers respectively from the left or "feminine" side, while Figs. 13 and 14 are corresponding feathers from the right or "masculine" side. The lighter appearance of the "cock" feathers of the right side is therefore not due in this case to relatively wider white bars, but must be attributed to the fact that they are older and consequently the black has faded to some extent, and to the difference in form and structure. Normal cock feathers are more elongate in these regions, and they differ

in structure from the hen feathers in that the firm web is relatively as well as absolutely narrower. In the feathers of the female the barbules interlock practically to the edge of the feather, making nearly the whole surface into a fairly firm web, but in the male the hackle and saddle feathers have a firm web only in the middle portion, practically the outer half[2] of the barbs being free of barbules and presenting a frayed appearance. These free barbs, together with the longer, pointed loose ends of the feathers cause the relatively rough, stringy texture of the hackle of the cock as compared with the smooth neck of the hen. The loose structure of the feathers in the former case, causing greater diffraction of the light, undoubtedly contributes to a lighter appearance of the hackle, irrespective of the broader white bars under normal conditions. Thus it will be noted that in "Kansas 1050," the masculine feathers assumed presumably as a result of the diseased ovarian condition were like cock feathers in respect to shape and structure, but still resembled hen feathers in the matter of width of bars, as shown by comparison with the new hen feathers grown after the new ovarian tissue was introduced.

The above would appear to support Spillman's interpretation of the normally wider white bars in the male as being due to homozygosis for the factor for barring. The form and structure of the feathers would thus be considered as secondary sexual characters dependent on the functioning of the sex gland; barring, however, being dependent directly on the genetic factor, would remain the same, whatever the sexual hormones. Although the failure of the ovary to function properly gave rise to a condition of internal secretion which led to the assumption

[2] Goodale's statement (1918, p. 292) that "the secretion of the ovary is necessary for the development of the barbules of the feathers of the dorsal regions since the barbules do not develop along the entire length of the barb in the male or in the capon" seems to be at variance with the above. If he means, as he seems to say in substance, that there are no barbules in the hackle, back, or saddle feathers of the male, his observations are certainly different from ours on Barred Plymouth Rocks. It is true that at the tip of the hackle feathers there are no barbules, but farther down they are to be found at the bases of the barbs, while just above where the fluff begins they run out practically to the extremities of the barbs. The statement that they are free on "the outer half" represents the average condition, or that found about midway from the fluff to the tip of the feather.

of male plumage with respect to shape and structure of feathers, the soma was still heterozygous for the barring factor and consequently the white bars in the "cock" feathers remained narrow as in the normal female. Davenport (1912, p. 17) classified sex-dimorphic characters into two classes: "(*a*) characters whose development is controlled primarily by determiners located in the sex-chromosomes and, (*b*) characters whose development is specially influenced or modified, probably by secretions of the sex-glands." In "Kansas 1050" we have both these classes represented in the same feather.

In the hackle and saddle feathers of the male the bars are less straight and regular than in the corresponding feathers of the female or in other parts of the plumage of either sex. In general there is a lagging of the middle portion of the bar, at the shaft, producing a more or less V-shaped bar instead of one straight across. This is illustrated in Figs. 9 and 10, and Figs. 13 and 14 show that the corresponding feathers on "Kansas 1050" had the same character. Thus it would seem that *shape* of bar, unlike *width*, is a secondary sexual character rather than sex-linked.

But while our case conforms to the interpretation given above, Pearl and Surface (1910, p. 53) state that in the cross between Barred Plymouth Rock and Cornish Indian Game "there is the same sort of sexual dimorphism in color pattern to be observed in the hybrids as in the pure Barred Plymouth Rocks," and specifically that "the male hybrids have narrower dark bars on the feathers especially in hackle and saddle feathers than do the females." These cross-bred males must of course have been heterozygous for barring, so that on Spillman's hypothesis they should have had the same width of bars as the hens. The fact that the crossbred males were darker than pure Barred Rock males does not alter this point, so that the matter cannot be considered as settled. It is of course possible that there were genetic factors carried by the non-barred breed (Cornish Indian Game) which might have had some modifying effect on the barring of the crossbred offspring.

"Kansas 1050" was killed and autopsied on July 28, 1917. Her behavior subsequent to the operation had not been notice-

ably different, but it was noted that for two or three weeks prior to the above date she had often been observed forenoons sitting in a nest-like depression in the straw. The notes also state that she had been a consistent "singer," keeping up a little crooning note a good share of the time.

The autopsy revealed the visceral organs normal except for an enormous tumor (Fig. 6) which filled practically all of the left side of the abdominal cavity and caused a marked abdominal protrusion. The tumor was for the most part highly vascular on the outside; it was roughly knobby, the larger knobs being several centimeters in diameter and dark purplish, almost black, in color; most of the smaller ones somewhat more reddish. The freshly removed tumor weighed 289 grams. The bird was not weighed before killed, but after the tumor was removed, most of the blood washed out and nearly all the feathers off, the carcass weighed 2,350 grams.

The tumor was attached at the normal site of the ovary, though the mesenteries were somewhat adherent to it at other points. There appeared to be no normal ovarian tissue remaining, nor, for that matter, was any trace found of the implanted pieces of ovary—at any rate anything which was positively identified as such.

The interior of the large knobs of the tumor exhibited a coarse trabecular structure, the trabeculæ being very dark and the matrix of a yellowish brown color. The smaller, softer knobs contained considerable blood, while the others were firm and hard. Greatest length of tumor, measured fresh, 13 cm.; breadth, 9.5 cm.

The oviduct had the appearance of that in a normal non-laying pullet.

The tumor was preserved and later referred for examination to Dr. C. H. Bunting, of the Department of Pathology at the University of Wisconsin. He has kindly given us the following description based on his study of the specimen. It seems advisable to publish this in full as a contribution to the knowledge of the anatomy of ovarian tumors in fowls.

"The specimen consists of a somewhat cruciform laked mass approximately 12.5 × 8.5 × 7.5 cm. in size. A distal small lake

approximately 2.5 cm. in diameter appears to be a subcapsular hæmorrhage. A large central lake appears to be made up almost entirely of large vascular channels and to be the site of extensive hæmorrhage. The proximal lakes show numerous large vascular channels separated by tissue, in the main rather translucent and glistening, but showing numerous small opaque areas.

"*Microscopical Study.*—Sections from the larger lake show many large channels filled with blood. Some of the larger of these are thrombosed; some of the thrombi are comparatively recent, others are old and show deposits of calcium salts in the hyaline material. The intervening tissue, which is small in amount, is necrotic.

"Sections from the proximal lakes show numerous vascular channels, few of which are thrombosed. These channels are separated by a considerable amount of connective tissue, which is in most places cellular, and apparently of recent growth. This tissue is in many places thickly infiltrated with lymphocytes and with numerous cells with eosinophilic granulation. There are recent small hæmorrhages into the interstitial tissue and evidence of old hæmorrhages, in the presence of hæmosiderin masses.

"In addition to the changes described there are throughout the intervascular tissue in these sections alveoli of various sizes, but usually small, solidly filled with cells of an epithelial (or endothelial) appearance. The cells vary much in size and appearance. The average cell has a nucleus of from 12 μ to 15 μ in diameter with distinct nucleolus and fairly heavy chromatin network. The protoplasm is abundant, granular, and shows a tendency to fatty degeneration. The cell outline varies. In some alveoli they are almost columnar, in others spindle-shaped, and in others their outline is difficult to determine as the cells are closely united into sheets or columns resembling a syncytium. While most of the vascular channels are lined by a delicate flattened endothelium, in some places they have a lining of high irregular cells of the character seen in the intervening alveoli. One further gains the impression that it is from such layers that the cells of the alveoli are derived. No evidence of ovarian structure is found in the sections.

"*Diagnosis.*—Vascular malignant tumor, apparently of endothelial origin."

The case of "Kansas 1050" falls naturally into the series described by Boring and Pearl (1918). The assumption of male plumage was obviously due to the degenerate and pathological condition of the ovary, and admits of the same interpretation that they give, namely that the general correspondence of the secondary sex characters to the primary sex organs "might be accounted for in accordance with the theory that the ovary forms an external secretion that inhibits maleness." No indication of a testis nor of testicular tissue was found in "Kansas 1050," though the search for it was not perhaps as careful as it might have been. The principal interest of our case seems to lie in the enormous size of the tumor, associated with apparent good health, the immediate effect on the incoming feathers of implanted ovarian tissue and the relations of form, structure and barring of the two sets of feathers.

Summary.

1. A purebred Barred Plymouth Rock pullet, designated as "Kansas 1050," which at first appeared normal, later assumed plumage much more resembling that of a normal male, and became much like a capon in general appearance.

2. This change in secondary sexual characters was obviously due to the development of a very large ovarian tumor.

3. Implantation of ovarian tissue from a normal pullet produced an immediate effect on new plumage assumed. New feathers grown in the few weeks succeeding the operation were definitely hen feathers.

4. The "male" feathers assumed early in life as a result of the pathological condition of the ovary, though like normal male feathers in shape and structure, resembled hen feathers in respect to barring. This distinguishes clearly the difference between *secondary sexual dimorphism*, as exhibited in the first instance, and *dimorphism caused through sex linkage*, as illustrated by the barring.

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NOTE.—While the foregoing paper was in press there came to our attention a paper by Albert Pézard on "Le conditionnement physiologique des charactères sexuels secondaires chez les Oiseaux. Du rôle endocrine des glandes génitales," Bull. Biol. de la France et de la Belgique, T. 52, Fasc. 1 et 2, pp. 1–176. This is a very interesting contribution to the literature of the genital glands in birds as endocrine organs and has an extensive bibliography, mostly of foreign references.

EXPLANATION OF PLATE I.

FIG. 1. Barred Plymouth Rock pullet "Kansas 1050" after assumption of male plumage.

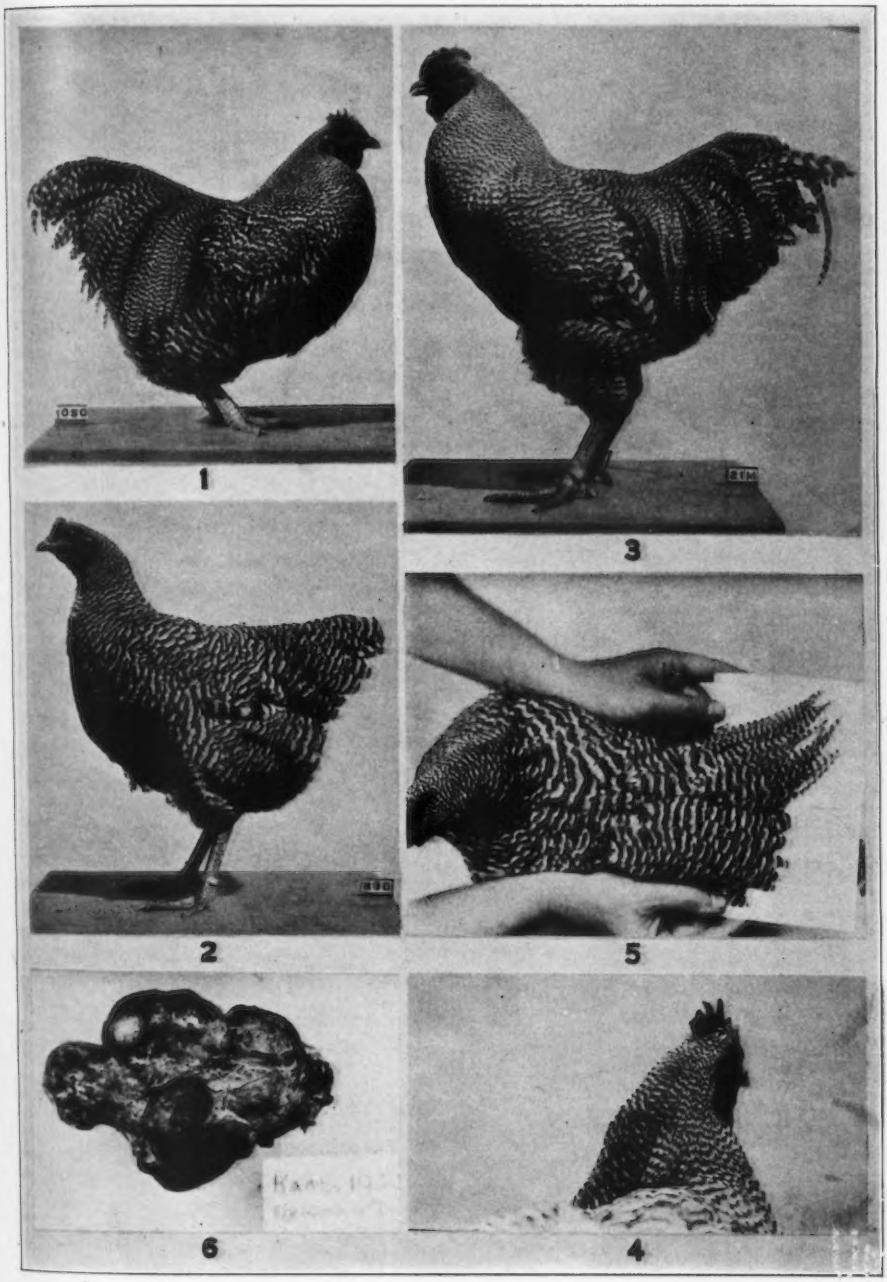
FIG. 2. Normal Barred Plymouth Rock pullet (Kansas Station No. 890), a flock sister of "Kansas 1050," and of approximately the same age.

FIG. 3. Normal Barred Plymouth Rock cockerel (Kansas Station No. 21M), a full blood brother of pullet 890.

FIG. 4. Plumage on back of neck of "Kansas 1050," photographed on July 28, 1917, nearly four months after implantation of ovarian tissue. The difference between the old "cock" feathers on the right side and the new "hen" feathers on the left side is plainly distinguishable.

FIG. 5. Dorsal view of "Kansas 1050," taken on July 28, 1917, showing the old and new plumage, particularly on the lower back.

FIG. 6. Ovarian tumor from "Kansas 1050."



L. J. COLE AND W. J. LIPPINCOTT.

EXPLANATION OF PLATE II.

Barred Plymouth Rock Feathers.

FIG. 7. Neck feather from normal female (Kansas Station No. 804).

FIG. 8. Cushion feather from normal female (Kansas Station, No. 804).

FIG. 9. Hackle feather from normal male (Kansas Station No. 104E).

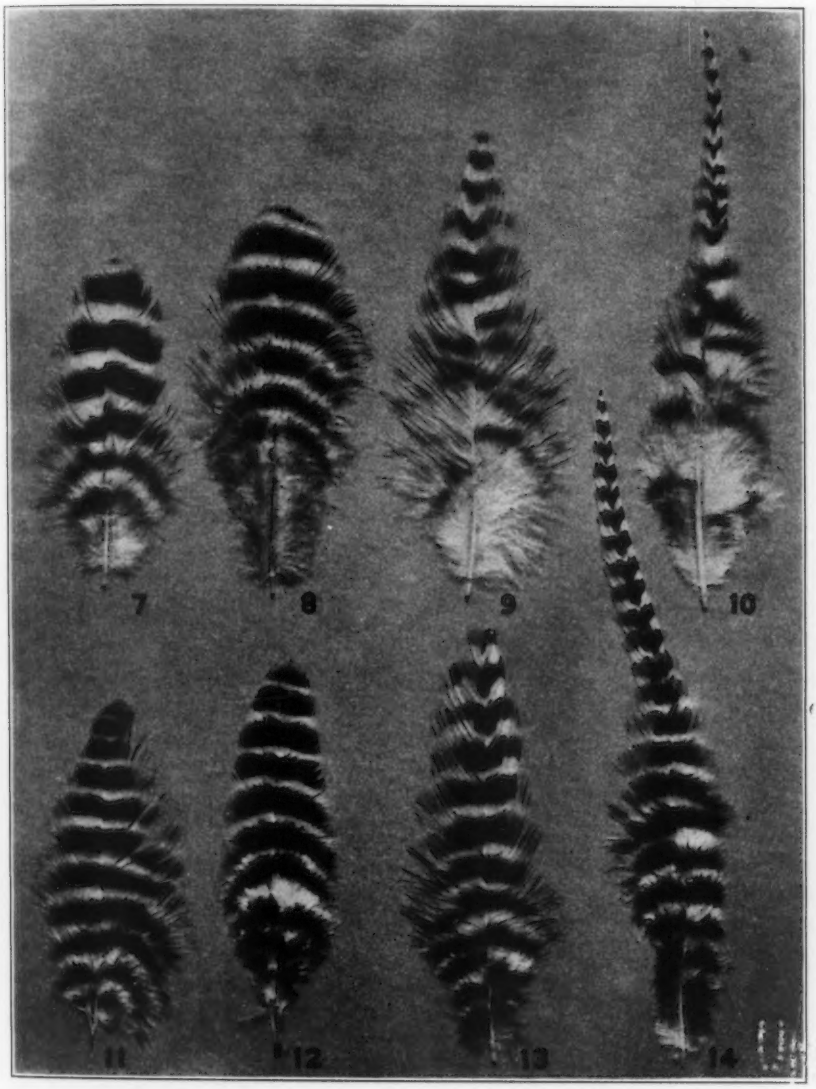
FIG. 10. Saddle feather from normal male (Kansas Station No. 104E).

FIG. 11. Neck feather from left ("hen feathered") side of "Kansas 1050," July 28, 1917.

FIG. 12. Cushion feather from left ("hen feathered") side of "Kansas 1050," July 28, 1917.

FIG. 13. Neck (hackle) feather from right ("cock feathered") side of "Kansas 1050," July 28, 1917.

FIG. 14. Saddle feather (from position corresponding to cushion) from right ("cock feathered") side of "Kansas 1050," July 28, 1917.



L. J. COLE AND W. A. LIPPINCOTT.

THE AXIAL GRADIENTS IN HYDROZOA.

I. Hydra.

C. M. CHILD AND L. H. HYMAN.

Department of Zoölogy, University of Chicago.

In the development and formulation of the general conception of the axial gradient conditions observed or experimentally induced in *Tubularia* and other Hydrozoa have played a part (Child, '15*c*, pp. 79, 91–2, 96–99, 128–137), but particularly during the last five years data concerning the gradient in these forms have been accumulating until now a point has been reached where the volume and variety of these data warrant their presentation.

While some of these data have been briefly considered, as noted above and in other papers, most of them have not as yet been recorded, and it is proposed to bring them together in connected form in this and two or three following papers. In the present paper the data on three species of *Hydra* are presented.

Methods and Material.

The gradients in *Hydra* have been studied chiefly by means of the direct or lethal susceptibility method[1] with cyanide in concentrations ranging from *m*/1,000 to *m*/10, ethyl alcohol 1–5 per cent., ethyl ether 1–3 per cent. and the dyes, neutral red, methylene blue and Janus green in wide ranges of concentration. With these agents the regional differences in susceptibility were determined, and the course of the gradient of disintegration and death in body and tentacles was observed under various conditions. Potassium permanganate, which has been found useful in many cases as a means of demonstrating the gradients as color gradients, has also been used to some extent (Child, '19), but is rather unsatisfactory because of the extreme contraction produced.

Observations have been made on the three common species of

[1] Child, '13*a*, *b*, '14*b*, '15*a*, *b*, chap. III., '15*c*, '16*a*, *b*, *c*, '17, Hyman, '16, 17.

Hydra, *H. viridissima* (*viridis*), *H. vulgaris* (*grisea*) and *H. oligactis* (*fusca*) and data on several hundred individuals have been recorded. The stages used include full grown asexual animals with and without buds, and all stages in the development of buds, and a few observations have been made on sexual individuals, animals developed from the regulation of pieces, and animals reduced in size by starvation.

The observations on *H. oligactis*, begun in 1913 by Child, have been repeated several times since on different stocks of animals and have been confirmed during the present year by Hyman. The observations on *H. viridissima* and *H. vulgaris* were first made by Hyman during 1918 and have been confirmed by Child. All important points concerning the gradients in the three species rest on observations made independently by both of us.

For the work on susceptibility, Syracuse dishes with covers of very thin glass are used. The animals are placed in the dish in a little water, or in certain experiments most of the water previously present is removed with as little disturbance as possible, the agent, freshly made up to the desired concentration, is added at once, or allowed to flow gently in from a pipette until the dish is quite full, and the dish is then covered with exclusion of all air bubbles. This procedure prevents loss by volatilization and makes it possible to examine the animals at all stages under a low power of the compound microscope, and the dish can be moved if some care is exercised, without disturbing the animals sufficiently to produce contraction.

Structural and Functional Specialization of Body Regions in the Three Species.

The four parts, tentacles, hypostome, column and foot present of course the same general characteristics in all three species, but there are certain features of the different body regions and certain minor differences between the species which are significant in relation to susceptibility.

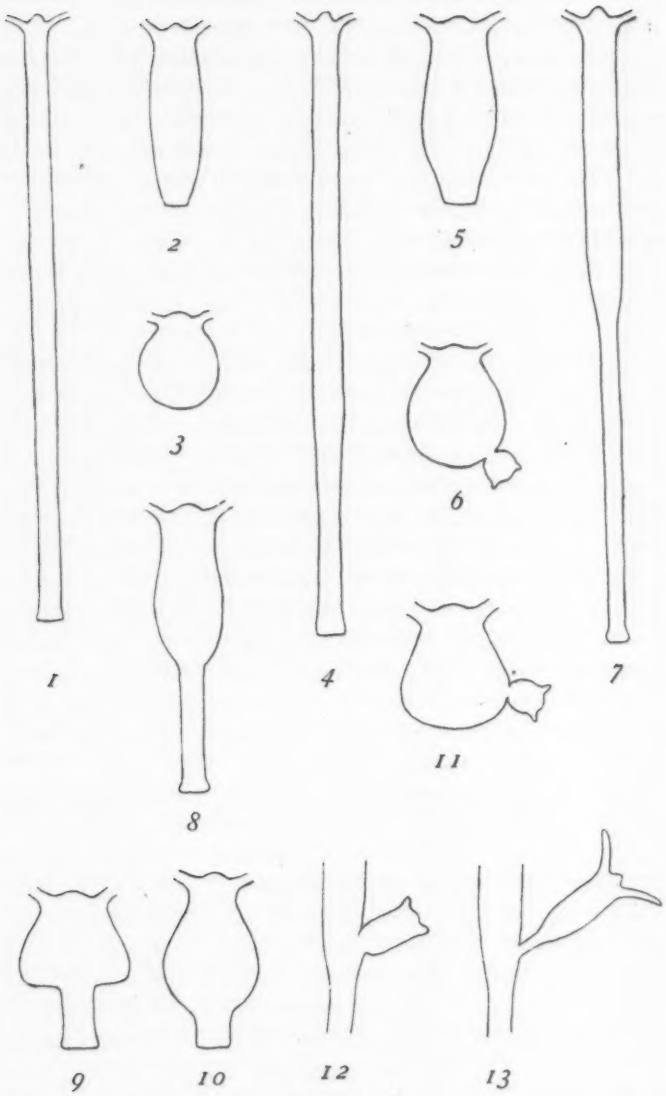
In the tentacles of all three species the capacity for elongation and contraction apparently decreases basipetally, but the tentacles of *H. oligactis* in the stocks with which we have worked, are much longer and capable of a much greater degree of exten-

sion and contraction than those of *H. viridissima* and *H. vulgaris*, which are much alike. The chief differences, however, are in the column. In all three species the basal region of the column including a third to a half the body length in ordinary degrees of extension is more extensile and contractile than the distal half or two thirds. In other words, all three species show at least a functional specialization of a more highly contractile basal region and a less highly contractile digestive region. Since this specialization is particularly significant in relation to susceptibility it will be convenient for present purposes to distinguish the basal region as the "stalk" from the digestive region or "body" in the stricter sense.

In *H. viridissima* this specialization is not usually accompanied by any marked degree of structural differentiation. The column of the fully extended animal is cylindrical and of practically equal diameter throughout its length (Fig. 1),[1] although there is usually a graded decrease in both the amount of nutritive substances and food reserves in the entodermal cells and of the symbiotic algæ toward the base of the column and consequently an increase in transparency in the same direction. In the various stages of contraction this basal region usually undergoes a greater degree of shortening than the more apical regions but is not marked off by any characteristic difference in form from the rest of the column (Figs. 2, 3). In extreme contraction such as is induced for example by strong chemical stimuli, the column may become spherical, or nearly so (Fig. 3).

In *H. vulgaris* a somewhat greater degree of differentiation of the stalk usually appears. In fully extended animals in good nutritive condition, the basal third more or less of the column is distinguishable not only by its thinner wall and greater transparency, the transition from the body to stalk being more abrupt than in *H. viridissima*, but the stalk is somewhat more slender than the body which often shows a slight increase in diameter basipetally to the beginning of the stalk region (Fig. 4). In a moderately contracted condition the stalk is likewise usually

[1] *H. viridissima* is of course much smaller than *H. vulgaris* and *H. oligactis*, but for conveneince in comparison all figures are drawn to such scale that corresponding stages of development of the three species are approximately of the same size.



FIGS. 1–13.

distinguishable as a region of smaller diameter than the body (Fig. 5) and in extreme contraction the body is not spherical but more or less urn-shaped with a somewhat flattened base, which represents the contracted stalk. Buds in *H. vulgaris* arise at or near the base of the body proper, and in the state of extreme contraction the position of the bud is at or near the border of the flattened base as indicated in Fig. 6. Since the bud serves as a landmark indicating approximately the boundary between body and stalk its position in Fig. 6 shows that there is in this contracted state a relatively greater decrease in length of stalk than of body.

Hydra oligactis shows a greater degree of differentiation of stalk and body than the other two species. In well extended animals in good nutritive condition the column is very distinctly marked off into two regions, the body proper, including the apical half or less of the column, with a larger diameter and a thick opaque entoderm, and the stalk, comprising the basal half or more of the column, with a smaller diameter very commonly only about half that of the body and a thin, highly transparent entoderm (Fig. 7). Moreover, the transition from body to stalk is in this form much more abrupt than in the other species. Fig. 8 shows a condition of moderate contraction, Figs. 9 and 10 greater degrees of contraction and Fig. 11 a state of extreme contraction induced by chemical stimulation. Since in *H. oligactis* as in *H. vulgaris* buds arise at or near the base of the body, the position of the bud in Fig. 11 indicates approximately the boundary between body and stalk and shows the greater contractility of the stalk.

The differences in diameter, general appearance and opacity between body and stalk become less marked with lack of food and in advanced starvation may almost or entirely disappear even in *H. oligactis*, and with less extreme starvation in *H. vulgaris*. On the other hand, individuals of *H. vulgaris* are not infrequently seen in which the entoderm of the digestive region is so thickened, apparently by nutritive reserves, that the form approaches that of *H. oligactis*, and occasionally *H. viridissima* shows some modification in this direction. But while the shape of the animal may vary widely with nutritive conditions, the

differences in specialization between stalk and body appear in general to be greater in *H. oligactis* than in the other two species.

Whatever its degree of development in full-grown individuals, the stalk is not present in young buds (Figs. 12, 14) nor in the earlier stages of the regulatory development of pieces of any of the three species, but is a secondary outgrowth from, or modification of the basal region of the column arising comparatively late in the development of buds (Fig. 13) and of pieces undergoing regulation. Not infrequently buds become detached from the parent before any considerable development of the stalk occurs, and in such cases the stalk develops later in the young, independently attached individual. In its early stages the stalk is very short, much shorter than the body and is not appreciably more contractile than other regions but both its length and its contractility increase until it is commonly somewhat longer than the body in well-extended animals of *H. oligactis* (Fig. 7) and shortens more than the body in extreme contraction (Figs. 10, 11).

The stalk is evidently then a more or less specialized region or organ arising at a late stage of development and functioning in connection with the sessile habit as an organ of extension and contraction. This being the case, it is also evident that the process of budding in hydra takes place at or near the basal end of the body proper, and in this connection it may be suggested that, while the specialization of the stalk is not so great as to prevent its dedifferentiation and regulatory development into a complete individual when it is physically isolated from the parent body, yet it is so much greater than that of the basal region of the body that the buds arise from the latter region rather than from the basal end or any other level of the stalk.

As regards behavior, both the tentacle and the column represent to a certain degree a relation of dominance and subordination. Under natural conditions the more distal regions of the tentacles must be more frequently stimulated than regions near the base, consequently contraction of the basal region of the tentacle is more likely to occur as the result of stimulation of a region distal to it than as the result of direct stimulation from without. While no extensive investigation of the problem has been undertaken simple experiments on mechanical stimulation with

H. oligactis indicate that the tentacle represents to some degree a gradient in physiological condition. While the reactivity of the tentacle varies and exact control of intensity of stimulation is not possible, the sensitiveness of the tentacle seems to decrease somewhat basipetally. Moreover, adequate stimuli near the tip usually induce contraction over some considerable portion or even the whole length of the tentacle, the contraction progressing basipetally, while similar stimuli near the base commonly induce merely a local contraction or bending of the tentacle at the region of contact with the concavity toward the exciting object. A more intense stimulation of the basal region may of course bring about contraction of the whole tentacle, but conduction of the excitation apparently occurs more readily basipetally than in the opposite direction.

Stimulation of one tentacle, if slight, may induce partial or total contraction of that tentacle only, if more intense, of all tentacles and contraction of all tentacles is usually followed by more or less contraction of the column.

As regards the column, contraction of the stalk usually occurs under normal conditions in response to excitation conducted from the tentacles or from some more apical level of the body. Certain degrees of stimulation produce contraction of only the more apical regions of the body or of the body and not the stalk, while more intense stimulation brings about contraction of both body and stalk. In animals partially anesthetized by alcohol, ether and other agents the decrement in conduction apparently increases, even when the body contracts vigorously, and the contraction can be seen to die out before reaching the stalk or in the anterior portion of the stalk. In such cases the stalk may become inactive before the body, not because it has lost its contractility, but because the excitation fails to reach it.

Both body and stalk are much less sensitive than the tentacles to direct contact stimulation. Contraction of the whole column can be induced by adequate stimulation at any point, as in the case of the tentacle, but stimulation of the more apical levels of the body induces, according to its intensity, contraction of only the more apical body region, of the whole body or of body and stalk. Here, as in the tentacle, a decrement in the transmitted excitation evidently exists.

The stalk, with the exception of the foot region, is under normal conditions less sensitive to direct mechanical stimulation than the body. It is often possible to puncture the stalk repeatedly with a needle, without producing any effect beyond a slight local contraction, or at the most some contraction of the whole stalk, but when the stimulus is adequate to produce more than a local effect, contraction of the whole animal usually follows. It appears then that because of the greater sensitiveness of more apical levels and probably also because of greater conductivity in the basipetal direction the physiological state at any level, so far as it is dependent upon excitation, is determined to a greater degree by the conditions in levels apical to it than by conditions at more basal levels. In other words the more apical levels are relatively dominant.

It should be noted, however, that the foot of the attached animal is rather sensitive to direct mechanical stimulation which deforms or stretches it, and the stalk likewise is sensitive to tension, whether produced by currents of water or through the tentacles, and after such stimulation of the foot or stalk, contraction of the whole animal usually occurs. Moreover, the stalk and foot of animals which have been forcibly detached are evidently in a different physiological condition from those of attached individuals, for the stalk of such animals performs searching movements to a greater or less extent, and the foot responds to contact by attachment except in conditions of extreme irritation. The stalk and foot apparently constitute a more or less specialized sensory and motor apparatus which under the usual conditions, *i. e.*, in attached individuals, is to some extent under the control of more apical regions, but which under certain conditions may respond independently or initiate the response of other parts.

The Axial Susceptibility Relations Under Various Conditions.

It is impossible to proceed far in the analysis of the susceptibility relations of hydra without becoming convinced that the contractile activity of any particular region is a factor in determining its susceptibility. The very direct and considerable influence of muscular contraction upon susceptibility is undoubt-

edly associated with the fact that the muscles of hydra do not constitute a special tissue, but are merely specially differentiated portions of the body cells or of certain of them. This being the case, there is every reason to believe, either that muscular activity in hydra is merely one expression of a general excitation of the cells concerned, or that excitation of the contractile portion of the cell brings about changes in the physiological condition of the cell as a whole, or it may be that both of these possibilities are realized under different conditions.

Whatever the nature of this relation between muscular activity and susceptibility it is necessary in analyzing the susceptibility relations to take into account the condition and activity of the muscular system. It is desirable to determine the susceptibility relations in the body, not only before the muscular system has developed, but also in fully developed animals under conditions of quiescence, moderate and extreme muscular activity and with experimental inhibition of muscular activity. Fortunately it is possible to investigate and analyze more or less completely all these different cases. Although the earlier embryonic stages are not available for work of this kind, the earlier stages of bud-development show little or no muscular differentiation and are to be regarded as essentially embryonic stages. Animals which have been attached by the foot and undisturbed for an hour or more are usually quiescent, while detached animals show a much greater degree of muscular activity. Moreover, different agents and different concentrations of the same agent produce very different effects upon muscular activity before death. Cyanide, for example, except in very high concentrations produces no appreciable excitation at any time before death and, on the other hand, inhibits muscular activity very gradually; it therefore affords favorable conditions for the analysis of the differences in susceptibility in non-motile buds and full grown motile animals and between attached quiescent, and detached animals.

All the other agents used produce more or less excitation accompanied by increased muscular activity in the earlier stages of their action. This condition of excitation may continue for hours in the lower concentrations of these agents, but is of course sooner or later followed by depression, disintegration and death. With

these agents then we have a combination of excitation and depression and in consequence of the differences in susceptibility, the degree of excitation and the length of the excitation period differ in different regions of the animals. Again, the degree of excitation and the length of the excitation period vary with concentration of the agent. In the higher concentrations of anesthetics, ethyl ether for example, and to a lesser degree ethyl alcohol, momentary excitation may be followed almost immediately by anesthesia and paralysis of the muscular system.

The dyes used, Janus green, methylene blue and neutral red, are all distinctly excitatory in action, Janus green being an intense irritant, even in very low concentrations, *e. g.*, 0.002 per cent., while methylene blue and neutral red are less irritating in their action. In general the higher the concentration of the dyes in solution, the more intense the irritant action, but in low concentrations the excitation increases to some extent with the accumulation of the dye in the tissues.

With this wide range of physiological and experimental conditions available, it becomes possible to control and modify susceptibility relations to a considerable extent and so to determine more definitely than would otherwise be possible various factors concerned.

In the following sections the susceptibility relations observed under these various conditions are described and analyzed. The figures although diagrammatic are all drawn from animals actually observed and indicate the degree of contraction of the body-regions in each particular case. In each figure the intact portions are indicated by continuous outlines, the disintegrated portions by dotted shading, and arrows beside the figure indicate the direction in which disintegration is progressing. Since the course of disintegration in the tentacles is almost invariably basipetal and, with only occasional exceptions, very similar in all tentacles of an individual, the figures show the tentacles only so far as is necessary to indicate their condition in each case. Parts of body or stalk already disintegrated are usually drawn only so far as is necessary to indicate the course of disintegration in figures of later stages.

While the stages and progress of disintegration indicated in

the figures concern primarily the ectoderm it should be noted that in well-fed animals the entoderm disintegrates at about the same time as the ectoderm, or in many cases somewhat earlier. Often disintegrated entoderm begins to flow out of the mouth before disintegration of the body ectoderm begins, and in the tentacles it can be directly observed that entodermal disintegration keeps pace with or follows very soon after disintegration of the ectoderm. In starved animals, however, the entoderm is distinctly less susceptible than the ectoderm, the difference between the two layers increasing up to a certain point with the progress of starvation. This difference between starved and fed animals is undoubtedly associated with the decrease in the metabolic activity of the entoderm in the absence of food. In the starving animal the functional activity of the entoderm must undergo very considerable decrease, while the functional activity of the ectoderm continues or may even increase with increasing "hunger."

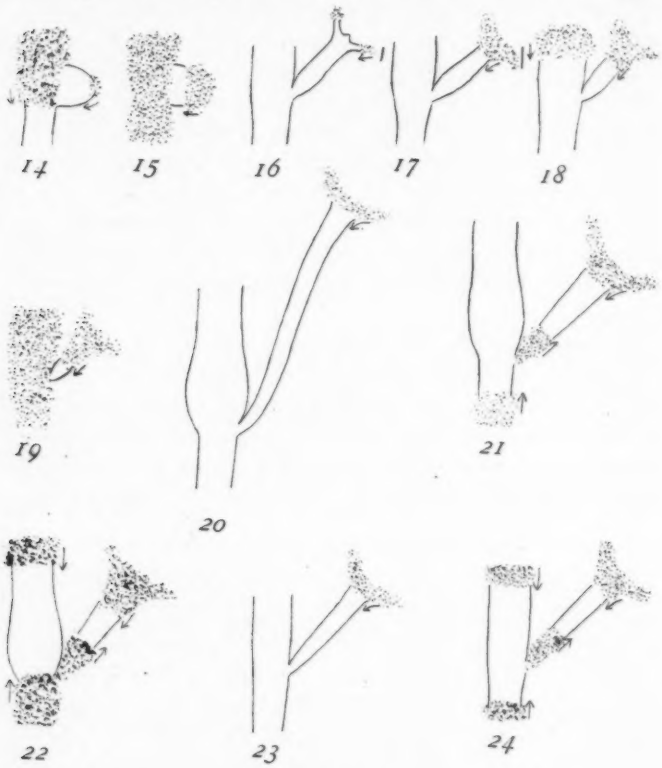
The mesoglœa does not disintegrate, and the general form of the animal is retained even after disintegration, except where there is considerable movement of the fluid, but the replacement of cellular structure by cellular detritus, and the increase in transparency, except in the dyes, make it possible to distinguish without difficulty the occurrence and progress of disintegration.

The Primary Gradient in Buds.

The earlier stages of buds before motor activity has developed to any marked degree show the same susceptibility relations in all three species and in all directly lethal concentrations of all agents used. The earlier stages in which the bud is merely a slight protuberance of the body wall, usually show no gradient distinguishable from that of the region to which it is attached, but sometimes even these early stages show the basipetal gradient characteristic of later stages (Figs. 14, 15). As the development of the bud progresses, however, a distinct disintegration gradient, basipetal with respect to the bud axis, makes its appearance (Figs. 14, 15) and in early stages of tentacle-development, but before the stalk has developed, the gradient is uniformly basipetal in both tentacles and column (Figs. 16–19) and under

the various experimental conditions, except in certain cases of strong contraction of the parent body or stalk to be mentioned below.

In still later stages, after the stalk has developed and the bud is nearly ready for separation, the course of disintegration varies according to conditions. Where the bud remains well extended



FIGS. 14–24.

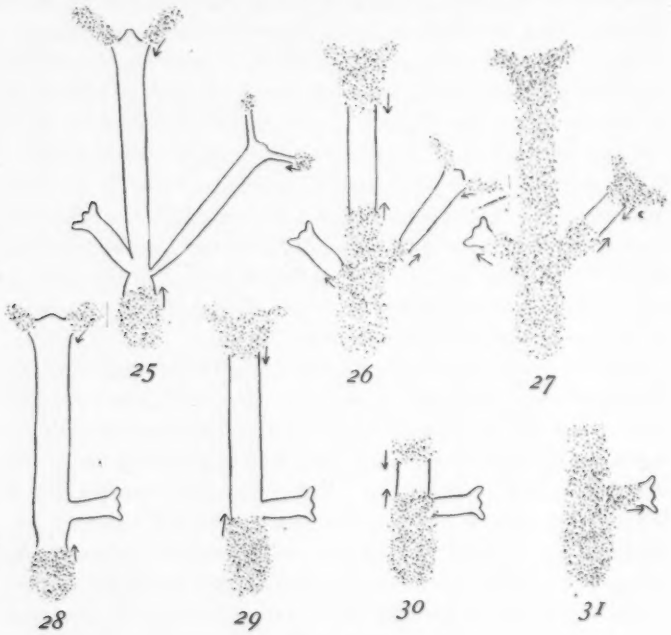
and quiescent the gradient is basipetal as in the earlier stages (Figs. 16–19), but where the stalk undergoes marked contraction in the susceptibility agent the stalk region appears as a region of high susceptibility, disintegration beginning in it as soon or almost as soon as in the hypostome region. In such cases disinte-

gration of the stalk may begin at the base and progress acropetally, or may occur at once throughout most of the length of the stalk, but it is evident in all such cases that the stalk has become a region of relatively high susceptibility. In Figs. 20–22 a case of this sort in *H. vulgaris* is shown. In the earlier stages of disintegration (in KNC) the bud remained extended (Fig. 20) but later underwent considerable contraction, the stalk region contracting more than the body, and soon after the contraction the stalk began to disintegrate (Fig. 21), and in later stages disintegration progressed basipetally in the body and acropetally but more slowly from the stalk (Fig. 22), so that the two gradients met near the base of the body. Figures 23 and 24 show a similar case in *H. viridissima*. Here the contraction occurred shortly after the parent and bud were placed in the KNC, but, as in the preceding case, the stalk appears as a region of high susceptibility, and two gradients of disintegration progressing in opposite directions result. These cases are merely examples of what has been observed by us repeatedly in all three species.

In Figs. 25–27 an animal (*H. oligactis*) with two buds, one less the other more advanced, is shown. Here the more advanced bud, which has already developed independent motor activity, shows contraction of the stalk and double gradients, as in the cases of Figs. 20–22 and 23, 24. The earlier bud, however, which is much less motile and less independent of the parent body, shows an acropetal instead of the usual basipetal disintegration gradient. This is one of the exceptional cases referred to above. It will be noted that the stalk of the parent is strongly contracted and shows a high susceptibility. The reversal of the gradient in the earlier bud results from the spreading or irradiation into the bud of the excitation connected with the contraction of the stalk. The more advanced bud is more independent, *i. e.*, its own gradient is more fixedly established and it is less intimately connected with the parent, so that is it less affected by contraction of the parent stalk, although the contraction and early disintegration of its own stalk may perhaps be the result of irradiation of the excitation from the parent. Figs. 28–31 show a similar case of reversal of a bud gradient in *H. vulgaris*.

Reversals of this sort in buds have been repeatedly observed

in *H. oligactis* and *H. vulgaris* and doutbless occur also in *H. viridissima*. Observations on the contraction of animals bearing buds in the earlier stages show very clearly that when marked contraction of the parent stalk or body or both occurs, some slight contraction of the bud may follow and in such cases progresses acropetally in the bud. In view of these facts, we believe that



FIGS. 25–31.

such reversals of bud gradients are to be interpreted as the expression of physiological relations which can be directly observed in the normal animals.

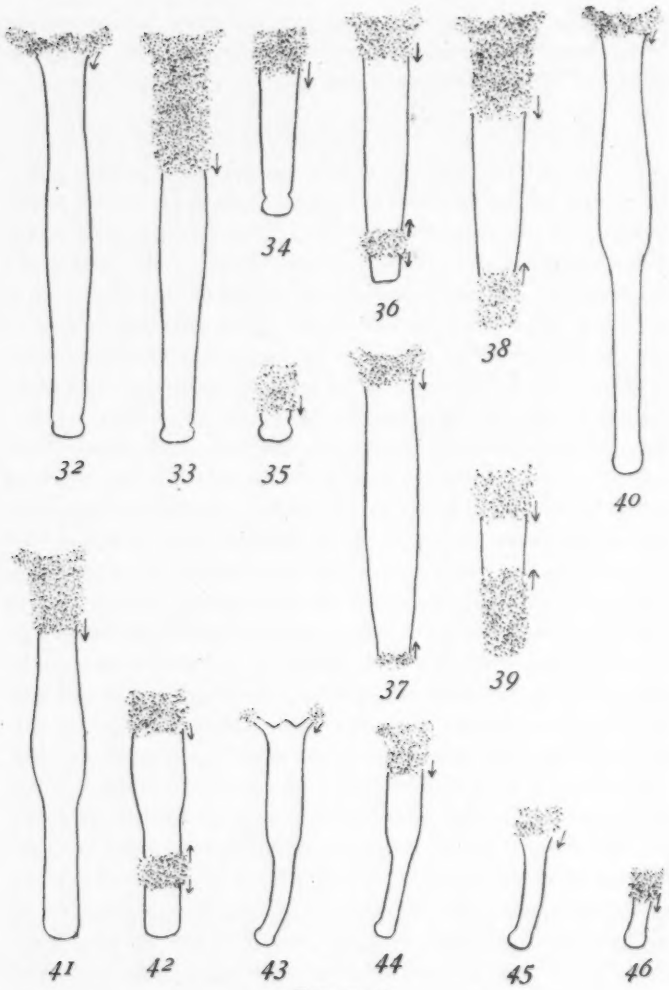
The results in all agents show that the primary gradient in the bud is basipetal, but that, even in the earlier stages, it may be modified or reversed by contractile activity in adjoining regions of the parent animal and in later stages by its own contractile activity. In the higher concentrations of anesthetics and in the irritant dyes certain other modifications of the susceptibility

relations in the later bud stages occur, but since they are similar to the modifications in full grown animals under the same conditions, their consideration is postponed to a later section.

The Gradients in Attached Quiescent Animals.

For present purposes "attached animals" are animals which have been left undisturbed in Syracuse dishes for several hours, usually over night, and are firmly attached to the glass of the dish. Animals attached to the surface film or to air bubbles are not included. After the preliminary period of attachment, most of the water is carefully drawn off, the agent added with a pipette and the dish covered without air bubbles, great care being taken to disturb the animals as little as possible. Since cyanide is the only agent among those used in this study which does not produce some considerable degree of excitation, it is employed in various concentrations from $m/1{,}000$ to $m/200$ with the most satisfactory results, where it is desired that the animals shall remain quiescent. The change of fluids is often accomplished without inducing any strong contraction, and if the animals contract at all, they usually extend again at once or remain at least moderately extended until death, unless otherwise disturbed.

Under these conditions individuals of *H. viridissima* and *H. vulgaris* show the primary gradient, disintegration progressing basipetally throughout tentacles, body and stalk (Figs. 32–35). In cases where the experiment is not entirely successful and any considerable degree of contraction of the stalk occurs, disintegration progresses basipetally as before in tentacles and body, but as disintegration of the hypostome begins, or somewhat later, disintegration also begins in the stalk somewhere above the foot and progresses in both directions (Fig. 36), or in some cases at the foot itself and progresses acropetally (Figs. 37, 38), death occurring last in or just apical to the basal region of the body (Fig. 39). Figs. 40–42 show a particularly clear case of this sort in *H. vulgaris*. Here the attached animal remained extended during the disintegration of tentacles and hypostome and the usual basipetal gradient appeared (Fig. 40), but at a later stage the stalk underwent considerable contraction (Fig. 41), probably in consequence of movements of the dish to and from the microscope



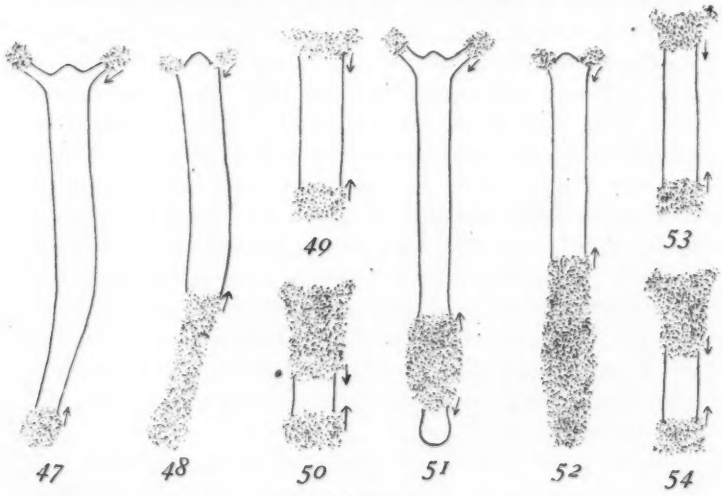
FIGS. 32–46.

stage, and soon after this contraction disintegration began in the stalk above the foot (Fig. 42) and progressed in both directions from this level with the usual final result.

H. oligactis differs markedly from the other two species in that attached quiescent animals in all cases observed by us, with

a single exception, show a secondary gradient or gradients in the stalk, and this secondary gradient extends further acropetally than in the other two species. In the first place, it is much more difficult to obtain attached quiescent individuals of *H. oligactis* for observation, for no matter how firmly attached and inactive they may be preceding the addition of the agent, most of them detach themselves either when the water is drawn off or when the agent is added. In many cases attachment occurs again in the solution within a few moments but is it preceded by more or less motor activity of the stalk. Moreover, in the individuals which remain attached the stalk apparently always undergoes some contraction.

In fact, probably the only completely successful attempt to obtain an attached quiescent individual for susceptibility determination is the case of the single exception mentioned above. This was a small animal, only recently separated from the parent body, which remained attached and quiescent during the whole period of observation and showed only the primary basipetal gradient (Figs. 43–46) like attached quiescent individuals of *H. viridissima* and *H. vulgaris*. Since this animal was not full grown and not far beyond the bud stage, and since the later bud stages



FIGS. 47–54.

often show a purely basipetal gradient, even after the stalk is distinguishable morphologically (p. 194), it is at least possible, that in this case the stalk had not attained complete specialization as a contractile region.

In all other cases of full grown attached individuals of *H. oligactis* more or less contraction occurred in the stalk, and the secondary gradient or gradients appeared according as the secondary disintegration began at the foot (Figs. 47–50) or above it (Figs. 51–54) and the acropetal gradient in all cases extended at least to the middle of the body (Figs. 50, 54) and often farther apically.

While these observations on the susceptibility of attached, quiescent animals indicate that the stalk of *H. oligactis* is more highly specialized than that of the other species, at least as regards its sensitiveness, they do not prove that it is permanently a region of high susceptibility.

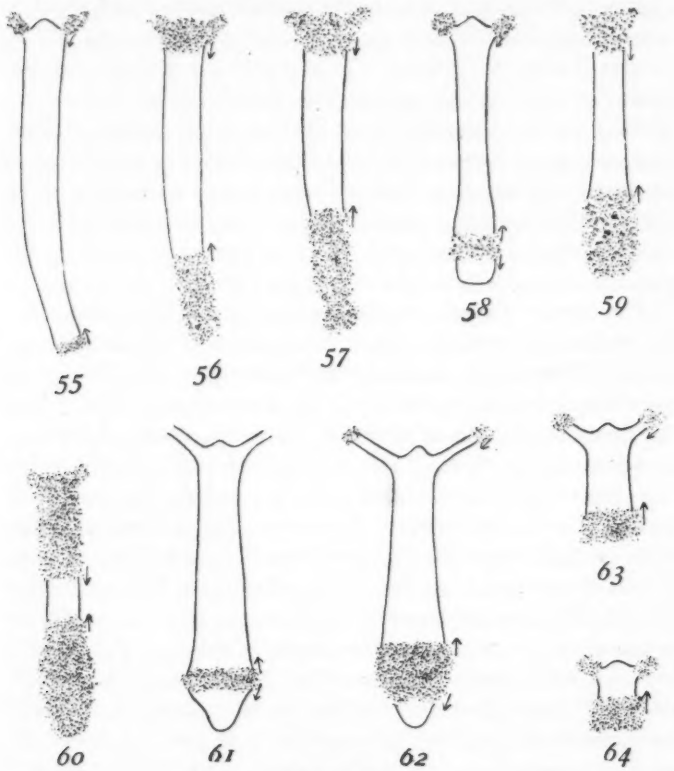
The Gradients in Detached and Recently Attached Animals Without Special Excitation.

In all three species the activity of the stalk is usually greater in the detached than in the attached animals even without any special external excitation, and the early appearance of disintegration at the foot (Figs. 55–57, *H. oligactis*) or in the stalk above it (Figs. 58–60, *H. vulgaris*) is the characteristic result as in attached animals with stalk contraction (Figs. 36–42 and 47–54). Here again KNC is the most satisfactory agent among those used, although the lower concentrations of anesthetics and dyes give the same results. In general the more active or more strongly contracted the stalk, the earlier it begins to disintegrate. In *H. oligactis* cases like 61–64 are not infrequently seen. Here the stalk is strongly contracted and the body progressively less contracted from the base apically, and disintegration begins in the stalk even earlier than the tentacles and progresses acropetally to the hypostome, *i. e.*, complete reversal of the gradient occurs, except in the tentacles. Such reversal has not been observed in the other species.

Rarely, however, detached individuals of *H. viridissima* and *H. vulgaris* remain quiescent with extended stalk and show the

primary basipetal gradient like attached quiescent animals (Figs. 32–35). Cases of this sort have not been observed in *H. oligactis*.

Animals which have been attached only a short time, *e. g.*, an hour or two, preceding exposure to the reagent, and animals which attach themselves in the solution, usually show gradients like those of the detached animals, *i. e.*, basipetal in tentacles



FIGS. 55–64.

and body and acropetal from the foot, or in both directions from some level of the stalk. In these cases there is usually more or less activity of the stalk for a time after attachment, and apparently the condition of excitation persists for some time after

muscular activity, though how long has not been determined. Sometimes, however, newly attached individuals of *H. viridissima* and *H. vulgaris* remain quiescent and well extended and show the primary basipetal gradient throughout.

Susceptibility in Relation to General Excitation, Anesthesia and Muscular Paralysis.

It was noted above (p. 191) that alcohol, ether and the dyes used produce primarily more or less excitation, as indicated by increased muscular activity. This condition is of course followed sooner or later by depression and muscular paralysis, and, according to the agent and concentration used, death and disintegration may occur under conditions ranging from extreme muscular relaxation to extreme contraction and rigidity. The susceptibility relations under these conditions afford further evidence that muscular activity is an important factor in the susceptibility of at least the ectoderm of hydra.

The degree of general excitation and the rapidity with which depression and paralysis follow excitation and therefore the regional differences in susceptibility depend to a considerable extent upon the concentration of the agents used. The lowest killing concentrations of alcohol and ether produce but little excitation, and in these disintegration and death usually follow the same course as in detached animals in cyanide (p. 200). Neutral red also, in the concentrations used, produces comparatively little excitation, and the susceptibility relations in this agent usually show no special modifications. Methylene blue produces a considerably greater degree of excitation and Janus green is an extreme irritant, and since these dyes accumulate in the cells, even when the external concentration is very low, a considerable degree of excitation occurs sooner or later, even in very low concentrations, but the higher concentrations bring about excitation more rapidly and to a more extreme degree than the lower. Only in extreme dilutions of these dyes is the course of disintegration like that in detached animals in cyanide.

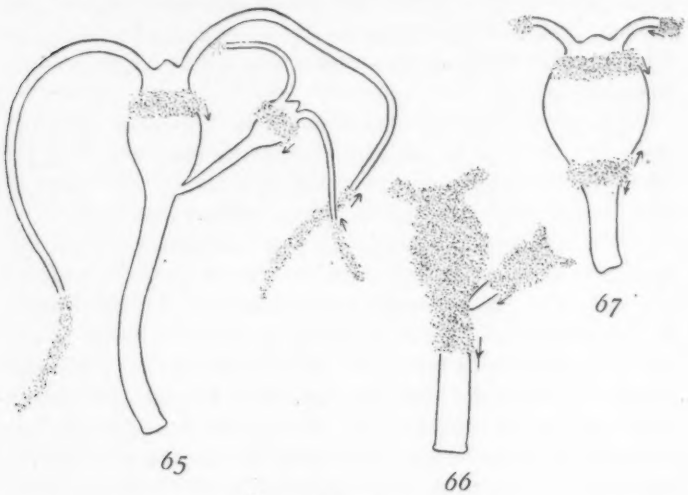
In all these agents, the earlier bud stages preceding the development of independent motor activity show, as already noted, the same basipetal gradient as in cyanide, except where the bud

gradient is partially or wholly reversed by the irradiation of excitation from the parent animal into the bud (p. 195). This primary gradient is then the same in all agents used. The late bud stages show essentially the same relations as the full grown animals and are considered with the latter.

Since the modifications of the primary gradient under consideration appear most distinctly and under the widest range of conditions in *H. oligactis*, they are first described as they occur in this species, and this description serves as a basis for comparison with the other species.

Experimental conditions and results with alcohol and ether are briefly as follows: the higher concentrations, ether 2–3 per cent., alcohol 3–5 per cent., are added rapidly to well extended animals in a little water and the dishes covered with exclusion of air. The tentacles and hypostome region are usually completely paralyzed in the extended condition before they are able to contract at all, the body begins to contract and usually succeeds in completing a strong contraction, but after that shows no further movement, and the stalk, which under normal conditions usually contracts later than the body, is either paralyzed before it can contract, or transmission of the stimulus from the body and excitation of the stalk are inhibited by the anesthetic before the stalk reacts. Fig. 65 shows an animal with advanced bud in the condition characteristic of this method of procedure. The tentacles are completely paralyzed, the body has contracted strongly and the stalk remains extended in both parent and bud. Under these conditions the disintegration of tentacles and hypostome is delayed as compared with that of the body, disintegration beginning in a zone just below the tentacle bases and in the tips of the tentacles at about the same time (Fig. 65). Disintegration progresses basipetally in both tentacles and body and also through the stalk (Fig. 66), the foot being the last region to disintegrate. The stalk usually undergoes a very slow decrease in length shortly before, or when disintegration begins (cf. Figs. 65 and 66). This is perhaps a response of the muscular portions of the cells to direct excitation by the reagent, or it may be an expression of the elasticity of the mesoglœa or other tissues, appearing after complete muscular paralysis.

In some cases in these concentrations of ether and alcohol and more frequently in somewhat lower concentrations, the stalk may undergo partial contraction at its apical end before paralysis occurs and in such cases the apical region of the stalk appears as a second region of high susceptibility (Fig. 67). In general, the lower the concentration of alcohol and ether used, the less rapid



FIGS. 65–67.

and less complete the paralysis of tentacles and hypostome, the greater the contractile activity of the stalk, and the closer the behavior both in motor activity and in the course of disintegration to the detached animals in cyanide.

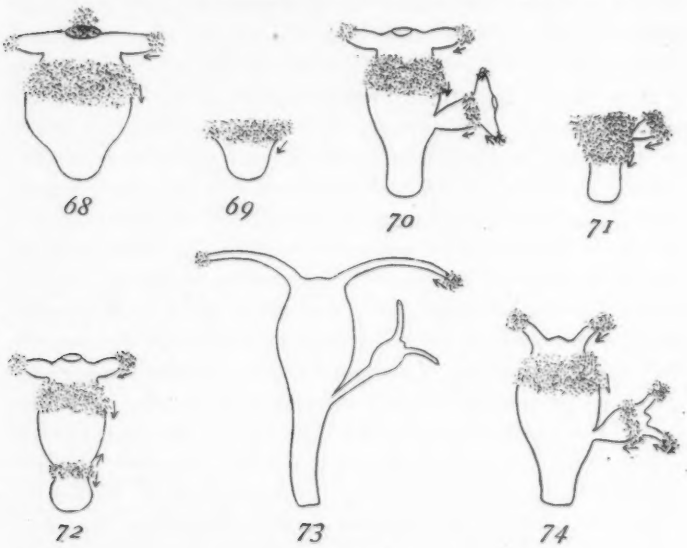
These experiments with alcohol and ether indicate first that inhibition of excitation and the resulting paralysis occur earliest in the tentacles and hypostome region. In concentrations where paralysis does not occur at once it can be seen that the paralysis progresses basipetally in the tentacles. In the column also the paralysis apparently progresses basipetally, although the fact that the body usually contracts before the stalk in the primary excitation may mask this relation in the higher concentrations. Apparently then the susceptibility as regards the paralyzing ac-

tion of these anesthetic agents shows a basipetal gradient like the primary gradient.

The second conclusion from these experiments is that disintegration and death are retarded in those regions which are paralyzed immediately or very early, as compared with those regions which show marked muscular activity before paralysis, so that disintegration may begin in the body below the tentacles (Fig. 65), and often also in the stalk, while the tentacles are mostly and the hypostome wholly intact.

The course of disintegration in methylene blue and Janus green is essentially the same as in alcohol and ether. In these dyes, however, the excitation is more marked and in the lower concentrations (methylene blue, 1/10,000 or less, Janus green 1/50,000 or less) may continue for hours, but is finally followed by paralysis and rigidity in a state of more or less extreme contraction. This paralysis and rigidity, which perhaps resembles muscle rigor, appears first at the tips of the tentacles and progresses basipetally in tentacles, body and stalk. The motor behavior in these dyes consists at first merely of elongation, contraction and other movements of all parts, but as staining progresses, the behavior comes to resemble more and more closely the disgorging reaction of normal animals. The mouth becomes permanently open, the hypostome flattened, the tentacles stand at right angles to the body, and the body itself undergoes repeated contraction, while the stalk is much less active and only very gradually attains a strongly contracted state. It may be noted that these dyes accumulate in the entoderm as rapidly as or even more rapidly than in the ectoderm, and the entoderm often begins to disintegrate before the ectoderm. To all appearances they irritate the entoderm and so give rise to the disgorging reaction. But whatever the exact physiological condition may be, these dyes produce relatively early paralysis of tentacles and hypostome, extreme activity of the body and less activity of the stalk before the final rigidity death and disintegration occur. As regards the course of disintegration, the result is much like that in alcohol and ether, disintegration beginning in the body below the tentacles and progressing basipetally while the tentacles are mostly and the hypostome wholly intact (Figs.

68, 69, Janus green 1/10,000). In Fig. 68 disintegrated entoderm is issuing from the mouth. Figs. 70 and 71 show another case, an animal with advanced bud in Janus green 1/200,000 approximately. In this low concentration the contraction is less extreme but the course of disintegration is the same as in the higher concentration. In these very low concentrations where the body contractions are less extreme, disintegration often appears at the anterior end of the stalk at about the same time as in the body (Fig. 72). In methylene blue the picture as regards both



FIGS. 68–74.

behavior and disintegration is essentially the same, but the degree of irritation with a given concentration is less than in Janus green. In these dyes, as in alcohol and ether, the relatively early paralysis of tentacles and hypostome, the great activity of the body and the less extreme activity of the stalk alter the regional susceptibility relations so that disintegration and death begin relatively early in the body and often in the stalk, as compared with tentacles and hypostome.

As regards *Hydra viridissima* the results in ether 2–3 per cent.

(Figs. 73–74) and in Janus green are essentially the same as in *H. oligactis*. The susceptibility of *H. viridissima* to alcohol, however, is very low, muscular activity continuing for 24 hours or more in 3 per cent., for 3–4 hours in 5 per cent. and for a minute or two even in 10 per cent. Alcohol produced but little excitation in this species and the disintegration gradient in alcohol is either basipetal throughout or double, as in detached animals in cyanide (pp. 200–202). The reason for the very low susceptibility to alcohol of this species we have not been able to determine. To cyanide and to ether it is somewhat more susceptible than *H. oligactis*. In methylene blue *H. viridissima* shows less excitation than *H. oligactis* and the course of disintegration is like that of detached animals in non-excitant agents. In Janus green, however, marked excitation occurs followed by paralysis beginning in the tentacles and hypostome and the course of disintegration is like that in *H. oligactis*. In other words *H. viridissima* shows this modification of the primary gradient only in those agents of this group which act most rapidly and most powerfully on *H. oligactis*.

H. vulgaris is apparently still less sensitive to the particular effects of this group of agents, and its motor behavior is less altered by them and only in Janus green, the most irritating of all, has the special modification of the gradient been observed. In this agent there is marked excitation ending in strong contraction and rigidity, and the course of disintegration is like that in *H. oligactis* (Figs. 68–72).

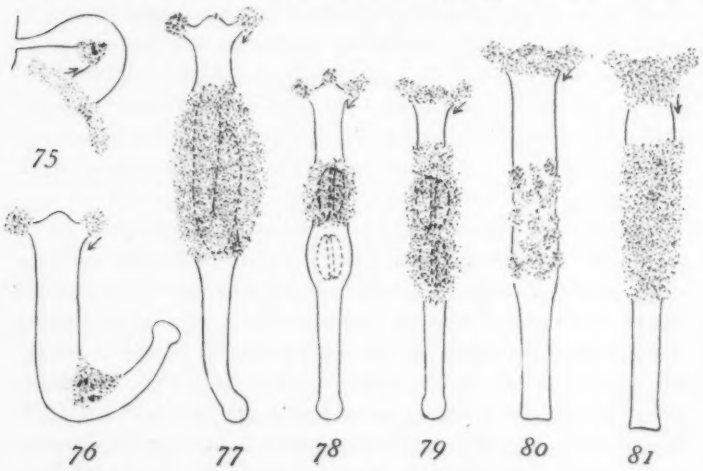
The fact that alcohol, ether, methylene blue and Janus green all produce the same modifications in the course of disintegration and death in *H. oligactis* suggests the possibility of a certain similarity in their action. All produce more or less excitation at first and later paralysis, though the period and degree of excitation differ widely. While the data are not adequate to permit definite conclusions, it seems evident from the observed facts that these agents act primarily upon the receptive or sensory and perhaps the transmissive mechanism of hydra and that the modifications of motor behavior and the course of disintegration are the indirect results of this action. We should expect the action of alcohol and ether to be of this character, methylene blue is a

more or less specific agent for nervous tissue in higher animals and the extreme irritation produced by Janus green indicates that it also affects this mechanism in some way. If this suggestion is correct, the basipetal course, first of excitation and second of paralysis, may indicate regional gradation in susceptibility in the receptive or nervous apparatus of hydra. Moreover, on this basis the differences between the three species suggest that this apparatus shows a higher degree of regional specialization in *H. oligactis* than in the other two species, and that *H. viridissima* is at least somewhat more sensitive or excitable than *H. vulgaris*.

Susceptibility in Relation to Unsymmetrical Contraction.

Usually all the tentacles of an individual behave in much the same manner in a given agent, but sometimes one or more tentacles contract more strongly than the rest. In such cases the more strongly contracted tentacle is usually more susceptible and disintegrates earlier than those which do not contract.

In cases also where the body becomes strongly bent and retains



Figs. 75–81.

that position, disintegration occurs earlier on the more contracted than on the less contracted side. Fig. 75 shows a case of this

sort in a late bud and Fig. 76 another in a full grown animal. Numerous other similar cases have been observed. It is of course possible that both the unsymmetrical contraction and the early disintegration indicate a local injury, although none was observed in these and other similar cases. Visible injuries to the body wall do become centers of early disintegration, undoubtedly in consequence of the excitation produced by the injury, and they may also bring about local contraction, though in many cases they do not. While local injury cannot therefore be entirely excluded as a factor in such centers of early disintegration, it seems evident that the early disintegration is the result of local excitation, whether this be due to injury or to muscular contraction or both.

LOCAL EFFECTS OF DIGESTIVE ACTIVITY ON SUSCEPTIBILITY.

When recently taken food is present in a particular region of the digestive tract a region of early disintegration appears corresponding at first to the region occupied by the food. Fig. 77 shows the case of a large individual of *H. oligactis* containing a recently ingested Chironomid larva as indicated. The region of the body in which the larva lies shows a very high susceptibility, both ectoderm and entoderm disintegrating at the same time as the tentacles and much earlier than other body regions.

Figs. 78 and 79 show another somewhat similar case. This animal, when isolated from the stock, had in its digestive tract the shell of an entomostracan, the soft parts of which were wholly or largely digested. An hour before the susceptibility determination entomostraca were placed in the dish with the animal and one was ingested. In the figure the shell of the first entomostracan is below the second newly ingested one. The body region containing the second animal which is in process of digestion shows a high susceptibility, both ectoderm and entoderm disintegrating at the same time as the tentacles (Fig. 78), while the lower region containing the animal which has already been largely or wholly digested is less susceptible, but still more susceptible than other regions of the body and stalk (Fig. 79). After the stage shown in Fig. 79 the disintegration did not extend from the regions concerned in digestion, but progressed basipetally from the hypostome and also began at the foot and progressed

acropetally, *i. e.*, the susceptibility of these regions was like that of many other detached animals.

Figs. 80 and 81 show the course of disintegration in an animal which contained a recently ingested chironomid larva when it was placed in cyanide, but disgorged the larva within the first five minutes. Although disintegration did not begin until an hour later, the region of the body in which the larva had been began to disintegrate as soon as the tentacles (Fig. 80) and was completely disintegrated before any other part of the stalk or body except the most apical region (Fig. 81).

These and numerous other similar cases observed indicate that the activity of the entoderm in digestion extends to the ectoderm of the region concerned. The relations already described between susceptibility and muscular contraction indicate that more or less transmission or irradiation of local excitations inducing contraction occurs, and these cases of high susceptibility associated with digestive activity indicate an irradiation to the ectoderm of the entodermal excitation produced by food. Probably no excitation is sharply localized in hydra.

The Color Gradients in Potassium Permanganate.

It has been found by one of us that the axial gradients in physiological condition in various plants and animals can be demonstrated as color gradients through the reduction of potassium permanganate to MnO_2 by the living protoplasm, the regions of high susceptibility and high oxidation rate reducing the permanganate more rapidly and so being stained more rapidly or more deeply by the MnO_2 (Child, '19). Some observations with permanganate have been made on all three species of hydra but the agent produces a great irritation even in very low concentrations ($m/50{,}000$) and the resulting contraction is so extreme that it is difficult to distinguish anything more than the general color gradations in full grown animals and later bud stages.

The earlier bud stages which possess little or no contractility show a distinct basipetal color gradient in permanganate, corresponding to the primary susceptibility gradient. In the tentacles also the color gradient is basipetal like the susceptibility gradient. Fully developed animals almost invariably detach

themselves at once in permanganate and always undergo extreme contraction. They show double color gradients, basipetal from the apical end and acropetal from the foot, but modifications of these gradients by contractile activity of stalk or body cannot be observed because extreme contraction occurs in all cases. As far as they go, however, these observations on color gradients agree with and confirm the data on susceptibility.

Susceptibility in Relation to Physiological Age.

The view has been advanced by one of us that a process of dedifferentiation and rejuvenescence is associated with agamic reproduction, and that the individuals arising from a part of a preexisting individual by process of budding, fission, etc., are physiologically younger than the individual from which they arose (Child, '15*b*, especially Chaps. VI., X.). In general the rate of the fundamental metabolic processes decreases with advancing senescence, and the agamically produced individuals show in their higher rate of growth, their higher direct susceptibility and greater capacity for acclimation, and in all cases thus far examined their higher rate of CO_2 production, that they possess a higher metabolic rate than the individuals from which they arose, and their morphological and cytological condition as compared with that of the parent individual also indicates that rejuvenescence has occurred.

If this view is correct, and if susceptibility to toxic agents is a criterion of physiological or metabolic condition, we might expect to find that the earlier bud stages of hydra are more susceptible than the animals from which they arise and that susceptibility decreases with advancing development.

As a matter of fact, however, the early bud is almost invariably less susceptible than the parent animal and the susceptibility of later bud stages is greater than that of earlier stages and is often equal to that of the parent. On the other hand, the "young" separate animal recently developed from a bud shows in general a higher susceptibility than the "old" full grown animal. As regards the early bud, Fig. 14 shows the usual condition, in which disintegration of the parent body occurs before that of the bud. In Figs. 18 and 19, a somewhat later bud stage, the disintegration

of the body is completed before that of the bud. In Figs. 20 to 22 the susceptibility of a late bud is about equal to that of the parent and in Figs. 23 and 24 the same is true. In Figs. 25–27 both the earlier and later bud are less susceptible than the parent, but here the susceptibility of the earlier bud is increased by irradiation of the excitation from the parent body. Fig. 28 shows another case where susceptibility of a rather early bud stage is less, even with gradient reversed by irradiation of excitation, than that of the parent. In Figs. 65 and 66, 70 and 71, 73 and 74 the susceptibility of advanced buds is seen to be about equal to that of the parent.

This apparent discrepancy in the results of the susceptibility method finds a very simple explanation in the total or almost total absence of motility in the earlier bud stages and its gradual development. In view of all the evidence presented above to show the influence of muscular activity upon susceptibility, it cannot be doubted that the development of motility in the bud compensates the effect of progressive development as regards susceptibility. The non-motile cells of the early bud, although physiologically younger than the cells of the late bud or the separate animal, are less susceptible than the cells which have developed a functional contractile mechanism. The susceptibility method does not of course distinguish between the intrinsic high metabolic rate of embryonic cells and the "functional" high rate of differentiated cells resulting from excitation. In these animals, where the muscles are merely portions of the cells and where excitation and muscular activity evidently alter the condition of the cells so greatly, the functional differences in metabolic rate usually overbalance the intrinsic differences associated with differences in physiological age.

When the bud has become a separate animal with fully developed excitability and motility, it is fairly comparable with the full-grown animal and at this time susceptibility is greater than that of the latter, *i. e.*, the functional characteristics being developed in both, the age differences now appear in the greater susceptibility of the younger animal.

This interpretation of the susceptibility relations of buds and parents and earlier and later stages of development is based on

wide observation by both of us, and agrees well with the other data on susceptibility. In a later paper it will be shown that similar relations exist in other hydrozoa.

The Susceptibility of Sexual Animals.

A few observations have been made on gonad-bearing animals with ether as agent. In all cases observed, the gonads are less susceptible than other regions of the animal. As in the case of early buds, the low susceptibility of the gonads is perhaps not a correct criterion of their physiological condition as compared with other parts of the body, for the reproductive cells are of course entirely separate from the muscular mechanism and are probably not very greatly affected by excitation and contraction in other cells. The number of sexual individuals available has been too small to permit definite conclusions concerning the comparative susceptibility of sexual and non-sexual animals, though the few data at hand indicate that sexual animals are in general less susceptible than non-sexual.

Susceptibility in Relation to Concentration.

Since the present paper is primarily concerned with other problems than those connected with concentration of agents, the data on concentration are incidental and presented merely to show approximately how rapidly the processes of disintegration occur in concentrations most commonly used. There is of course some individual variation in susceptibility, doubtless dependent on uncontrolled factors such as nutritive condition, physiological age, motor activity, etc., and the course of disintegration differs under different conditions, as already described. A few characteristic data for large, well fed, asexual individuals of *H. oligactis* at temperature 20°–22° C. are tabulated for illustration and comparison.

The concentrations given in the table for KNC, alcohol, and ether are of course only approximate. All solutions of these agents were freshly made in every case and loss from volatilization was prevented as far as possible. As regards methylene blue and Janus green, the tabulated data are all from solutions of the same sample of each. Different preparations of these

TABLE.

Agent.	Concentration.	Time in Hours from Beginning of Experiment.	
		Beginning of Disintegration.	Completion of Disintegration.
KNC	*m*/1,000	2– 3	10–18
	m/400	1 ±	6– 9
	m/200	½ ±	4– 6
	m/20	1– 5 minutes.	¼–½
	2 per cent.	Usually not directly lethal within 24 hrs. except for tips of tentacles.	
Alcohol	3 per cent.	5– 8	18–24
	4 per cent.	3 ±	8–10
	5 per cent.	1 ±	4– 6
Ether	1 per cent.	½– 1	3– 5
	2 per cent.	¼ ±	½ ±
	3 per cent.	5–10 minutes.	¼–½
	0.0005 per cent.	12–16	22–24
	0.01 per cent.	8–12	18 ±
	0.02 per cent.	6– 8	10–18
	0.0002 per cent.	2 ±	18–20
	0.002 per cent.	1 ±	4 ±
	0.01 per cent.	½ ±	2 ±
	0.05 per cent.	at once	¼

dyes showed some difference in degree of toxicity. The plus or minus sign following the figures denoting times indicates that the exact time was not observed. The data for neutral red are not tabulated because the gradual precipitation of the dye in aqueous solution makes it impossible to maintain a constant concentration, but in a concentration originally approximately 0.005 per cent. of the sample used disintegration began after 1–2 hours and was completed after 6–7 hours.

Young, recently separated animals disintegrate in half to two thirds the times given in the table, animals regenerated from pieces resemble the young animals in susceptibility, and in starved animals the ectoderm is more susceptible than in well-fed animals while the entoderm, being in large measure functionally inactive, is usually less susceptible than in well fed animals.

In general the susceptibility of *H. viridissima* and *H. vulgaris* to a given concentration of a particular agent is somewhat greater than that of *H. oligactis*, but the low susceptibility of *H. viridissima* to alcohol (p. 207) is apparently an exception to this rule.

Comparisons between the species are best of limited value because various physiological conditions play so large a part in determining the susceptibility of the individual.

Discussion.

The data show first, that a primary axial gradient in susceptibility exists in the three species of hydra, with the region of highest susceptibility at the apical end, and second, that this gradient is variously modified by the functional activity and particularly by muscular contraction in the various regions. If the view advanced in various papers (see references, Child, Hyman) and supported by various lines of evidence, that susceptibility is in a general way a criterion of metabolic and physiological condition, is correct, the facts indicate that there is in hydra, as in other axiate organisms examined, an apico-basal metabolic gradient, with region of highest metabolism at the apical end. It is perhaps scarcely necessary to repeat once more that the differences in metabolic rate are only one feature of such a gradient. Associated with them are of course differences in protoplasmic condition of various sorts. The important point is that the apico-basal axis in hydra, like the axis of other organisms, appears as a gradient in relation to the action of external agents. Discussion as to what constitutes the primary feature of such a gradient is at present of little value, since metabolism and protoplasm are always associated in active living organisms. Likewise, the question how each particular agent acts on protoplasm is of minor importance for the general conception.

As in other axiate forms, the primary gradient corresponds to a relation of physiological dominance and subordination, the region of highest susceptibility being to a greater or less degree a region of initiation and control. In hydra this is true, both of the tentacle and of the column. The stalk represents physiologically a more or less specialized contractile region secondarily developed from the basal portion of the body, but the primary gradient is altered by the contractile activity of the stalk, not by its mere presence, except possibly in *H. oligactis*, though even here the high susceptibility of the stalk observed in most cases is apparently associated with more or less muscular activity.

Whatever the terms in which we may finally define the axial gradient, its existence and significance cannot be doubted. In hydra, as in other forms examined (see references, Child, Hyman), it is the earliest indication of the body axes, it is certainly associated with quantitative differences in metabolic and general physiological condition along its course, and physiological dominance and subordination, as well as local specialization and differentiation exist or arise in definite relation to it. In the light of all the known facts, the only possible conclusion is that the gradient is the simplest physiological expression of the axial relation in organisms. In other words, this axial relation in its simplest terms is a quantitative gradation in the condition of living active protoplasm.

The modifications of the gradient in hydra by contractile and digestive activity are significant in that they constitute further evidence for the value of the susceptibility method as a means of investigating metabolic condition or "physiological state" in simple organisms. Since the contractile mechanism in hydra is merely a part of the body cell, excitation and muscular contraction affect the whole cell to some extent, and the altered condition of the cell appears in its altered susceptibility. Similarly the spreading or irradiation of excitation with both local muscular contraction and local digestive activity also appears in the susceptibility relations of different regions. The simplicity and diffuse character of the organization of hydra make it of particular interest in this respect, because each excitation produces a more or less diffuse effect.

The experimental data indicate further that a greater degree of excitation and change in physiological state is associated with the muscular activity which brings about contraction of tentacles, body or stalk, than with that concerned in their extension. Extension is doubtless due, at least in large part, to the activity of transverse or circular muscle fibers, but the process is much less rapid and less vigorous than that of contraction and occurs in the absence of strong external excitation, while contraction is induced by external excitation. Apparently the longitudinal muscles are more numerous, or more powerful, or their contractile activity is associated with more intense excitation, and it is this

activity, or conditions associated with it, which play the chief part in modifying the primary susceptibility relations.

On the basis of studies of oxygen consumption and susceptibility in protozoa, E. J. Lund ('18*a*, *b*, *c*) has criticized the conclusion that susceptibility to cyanide is a measure of rate of oxidation or of metabolism. He has found, first, that *Paramecium* in cyanide shows no decrease in oxygen consumption until cytolysis or disintegration occurs, and second, that in starving *Paramecia* oxygen consumption decreases, while susceptibility increases.

In the absence of any attempt to account for the fact that his results apparently disagree completely with current theories of cyanide action, and of convincing evidence that his experiments are controlled with sufficient care, his negative results require further confirmation. But, even assuming that his experimental data are correct as they stand, he has apparently failed to recognize an essential point, viz., that susceptibility as measured by the progress of death and disintegration concerns primarily the ectoplasm (Child, '14*b*) or the body-surface and body-wall (Child, '15*b*, p. 75). Statements to this effect have been made repeatedly in the work on susceptibility.[1] It is highly probable that in *Paramecium* the oxidation in the thin layer of ectoplasm is only a very small fraction of the total oxidation, and that, therefore, the decrease in oxygen consumption resulting from the action of cyanide on the ectoplasm is not sufficient to appear with certainty in the total oxygen consumption, with the small amount of material used in his experiments. As soon as the ectoplasm begins to disintegrate and the entoplasm is directly exposed to the action of the cyanide, Lund finds a very marked decrease in oxygen consumption, *i. e.*, KNC does decrease oxidation at least in the entoplasm in his experiments. The susceptibility method, on the other hand, shows only the conditions in the ectoplasm. In short, Lund's data concerning the effect of cyanide on oxygen consumption in *Paramecium* are negative and inconclusive, except as regards the entoplasm, where they are positive and in agreement with current theory. They do not, therefore, justify

[1] Lund's attention was called to this point in personal correspondence soon after the preliminary report of his work appeared (Lund '18*a*).

his conc'usion concerning either the action of cyanides on oxidation in *Paramecium*, or the results of the susceptibility method. In papers soon to be published we shall show that KNC produces a very marked decrease in both oxygen consumption and carbon dioxide production in *Planaria*.

His conclusions concerning oxygen consumption and susceptibility during starvation indicate that he has failed to take into account a second important fact, viz., that metabolic conditions in ectoplasm and entoplasm may be different and may change more or less independently. As a matter of fact Lund's data on starvation metabolism in *Paramecium*, as far as they go, are in full agreement with, though much less complete than, the results of our own studies made during the last two years on starvation metabolism in *Planaria*, but not yet published. While extended discussion and criticism along this line are postponed until publication of our own results, it may be noted here that the apparent discrepancy between susceptibility and metabolism appears in *Planaria* as well as in *Paramecium*, but as regards *Planaria*, it has been possible to demonstrate that it is only apparent. In well fed animals the metabolism in the alimentary tract is a very large part of the total, and during the early stages of starvation this metabolism at first decreases very rapidly as the alimentary tract becomes less and less active functionally. During this period, however, the functional activity and metabolism of the surface and body wall continue and the susceptibility method shows that the susceptibility of the body wall remains unchanged or increases slightly, but it also shows that the susceptibility of the alimentary tract as compared with that of the body wall decreases to a very marked degree. In other words, if we take into account the susceptibility of the alimentary tract as well as that of the body wall, which the data published in earlier papers did not do (Child, '14*a*, '15*b*, Chap. VII.), the discrepancy between the results on susceptibility and those on metabolism disappears.

In the more advanced stages of starvation, concerning which Lund presents no data, perhaps because *Paramecium*, being a single cell, dies before it reaches such stages, when all food and all reserves are gone, and the animal is living upon its own proto-

plasmic substance, the total metabolism begins to increase, and sooner or later attains a higher level than in the original animal. During this period susceptibility of both body wall and alimentary tract increases, but the susceptibility of the latter always remains lower as compared with that of the body wall in the starved than in the fed animal.

The apparent discrepancy between the susceptibility and metabolic methods results simply from the fact that the alimentary tract, and doubtless the entoplasm in *Paramecium* soon becomes practically inactive functionally in starvation while the body wall does not. Consequently total metabolism may decrease while susceptibility of the body wall increases or remains unchanged, but later both the susceptibility of body-wall and alimentary tract and the total metabolism increase.[1]

This discussion is sufficient to make it at least highly probable that Lund's conclusions result rather from inadequate analysis of his own experimental data in the light of the known facts, than from any real discrepancy between the results obtained by the susceptibility method and by direct measure of oxidation. He has failed to take account of two very essential facts: first, that ectoplasmic or body-wall oxidation is very far from being total oxidation and second that the rate of oxidation in ectoplasm and entoplasm or in body-wall and alimentary tract may vary independently, that of the alimentary tract or the entoplasm undergoing marked decrease in starvation.

Attention must also be called to the fact that the susceptibility method does not, as Lund asserts, depend upon the assumption that cyanide is a specific inhibitor of oxidations, for the regional differences in susceptibility, the susceptibility gradients as well as the differences with age and other conditions, are the same with many other chemical and physical agents and conditions in proper concentration or intensity, including for example high and low temperatures and lack of oxygen and perhaps external agents and conditions in general, as they are with cyanide.

The anesthetics and dyes used in the study of susceptibility in hydra, for example, give the same results as cyanide except where

[1] Lund was also informed in personal correspondence concerning our results on *Planaria* and the reason for the apparent discrepancy between susceptibility and total metabolism was pointed out to him.

special irritating or paralyzing effects of those agents are concerned, and the modifications in susceptibility only serve to show all the more clearly the close relation between susceptibility and metabolic condition.

The dyes were used in these experiments simply as a means of further analysis of the action of external agents. Since they possess color, it is possible to observe directly their penetration into the cells and to see that they enter all cells readily. It is certain that neutral red and methylene blue kill primarily from within the cell rather than by destroying or altering its surface first as perhaps some other agents do. They are adsorbed or otherwise accumulated by certain constituents of the cell, and it is of great interest to find that the general susceptibility relations with these dyes are essentially similar to these with other agents, except where special irritating or other effects are concerned. The dyes enter all cells, but they kill certain cells earlier than others because the cells differ in some way in physiological condition. Judging from their irritating effect, they probably increase metabolism to a very marked degree, at least at first, yet where the irritation is not too extreme, the death gradient is the same as in cyanide and other agents. No assumption concerning the specific action of cyanide is necessary as a basis for the susceptibility method, though it is quite true that the conception of the action of cyanide current among physiologists has been adopted and applied in connection with the method. The value of the susceptibility method rests not upon assumption but upon certain facts of observation and experiment: first, that in the susceptibility of protoplasm to many, perhaps to all, external agents a certain uniformity of relation appears as regards region of body, stage of life cycle, nutritive condition, etc.; and second, that this uniformity is in some way associated with and dependent upon the fundamental metabolic processes in the protoplasm and may be altered by changes in the rate of these processes.

Both E. J. Lund in his paper on starvation in *Paramecium* ('18*c*) and B. L. Lund in a paper on the susceptibility of *Paramecium* and *Didinium* to KNC ('18), which confirms earlier work of one of us (Child, '14*b*), maintain that susceptibility is

dependent upon permeability and that permeability is independent of rate of oxidation. The chief basis for this view is the work of E. J. Lund on starvation, and it has been pointed out that his conclusions depend upon an interpretation of his data which in the light of the known facts is almost certainly incorrect: if this is the case, the whole contention of these authors fails. There are, moreover, certain data on susceptibility, such, for example, as the results with the dyes in hydra and other forms, which cannot be interpreted in terms of permeability in any physical sense, and other data on susceptibility to lack of oxygen, which show that other factors than permeability are concerned in susceptibility, are soon to appear. As one of us has stated elsewhere (Child, '19), the most important question in this connection is the question of the nature of what current terminology calls permeability. Extended discussion of this point is postponed to another time, but it may be noted that no purely physical conception of permeability accounts for all the known facts, that according to all the evidence, the surface as well as the interior of the cell is alive and the seat of metabolic reaction, and that susceptibility, and even permeability itself, are dependent, not merely upon the physical characteristics of the surface layers as a membrane, but also, at least in part, upon this living, chemically active condition. Apparently for the Lunds permeability is some condition at the cell surface completely dissociable from the metabolic condition of the protoplasm, but their own evidence that such a condition exists is at least far from conclusive as we have shown, and other adequate evidence is difficult to find.

Summary.

1. A study of the susceptibility of three species of hydra, *H. viridissima* (*viridis*), *H. vulgaris* (*grisea*) and *H. oligactis* (*fusca*) to various agents shows that a gradient in susceptibility exists along the apico-basal axis of the body and in each tentacle.

2. This susceptibility gradient is primarily a basipetal gradient, *i. e.*, the susceptibility decreases from the apical region basipetally and after the tentacles arise, in each tentacle from the tip to the base. This primary gradient appears in the earlier bud

stages, in quiescent attached individuals of *H. viridissima* and *H. vulgaris* at all stages and in young quiescent individuals of *H. oligactis*.

3. This primary gradient is modified in various ways by regional differences in functional activity and particularly by the special activities of the stalk, a more or less specialized organ which develops at a relatively late stage from the basal body region.

4. Regional differences in the contractile activity of the muscular apparatus produce the most conspicuous modifications of the susceptibility gradient. Such modifications can be produced by detachment of the animals, which leads to special activity of the stalk, by the general excitatory or special irritant action of certain agents, such as the dyes used, and by certain combinations of excitation and paralysis with anesthetics.

5. Alterations of the primary susceptibility relations are produced, not only by regional differences in muscular activity, but by regional differences in digestive activity.

6. The very direct and marked effect upon susceptibility of regional and local differences in muscular and other functional activity results from the simple and diffuse organization of hydra. The muscle, for example, is a part of the body cell and the whole cell shares in the excitation connected with muscular contraction, and the facts indicate that most or all local excitations spread or irradiate to a greater or less degree to adjoining regions.

7. In stages after the development of motor activity susceptibility decreases in general with advancing physiological age, but the bud in stages preceding the development of motor activity is less susceptible than later motile stages.

8. The differences in the susceptibility relations of the three species under various conditions suggest that of the three *H. oligactis* possesses, as its structural and functional characteristics indicate, the highest degree of regional specialization.

9. The facts confirm previous work in that they indicate the existence of a relation of some sort between susceptibility and metabolic rate. Within the ranges of concentration of the agents used, susceptibility evidently varies in general directly with metabolic rate, or with the rate of energy-liberating metabolism.

If such a relation exists, the axial gradient is a gradient in physiological condition, of which rate of fundamental metabolic reactions is one feature.

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